

11-15-00

A

EXPRESS MAIL CERTIFICATE

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated below and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Typed or Printed Name	Mather OTTS	Express Mail No.	EL 563 650 743 US
Signature	<i>Mather</i>	Date	11-13-00

UTILITY  
PATENT APPLICATION  
TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Atty Docket No. IRVN-007CON

First Named Inventor Gatanaga, et al.

Title: Factors Affecting Tumor Necrosis Factor Receptor Releasing Enzyme Activity

11/13/00  
JC515 U.S. PRO  
09/712813

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

Address to: Assistant Commissioner for Patents  
Box Patent Application  
Washington, D.C. 20231

1. ☒ Fee Transmittal Form
2. ☒ Specification Total Pages 100  
(preferred arrangement set forth below)
  - Descriptive title of the invention
  - Cross Reference to Related Applications
  - Statement Regarding Fed sponsored R & D
  - Reference to Microfiche Appendix
  - Background of the Invention
  - Brief Summary of the Invention
  - Brief Description of the Drawings (if filed)
  - Detailed Description
  - Claim(s)
  - Abstract of the Disclosure
3. ☒ Drawing(s) (35 USC 113) Total Sheets 05
4. ☒ Oath or Declaration Total Sheets 02
  - a. ☒ Newly executed (original or copy)
  - b. ☐ Copy from a prior application (37 CFR 1.63(d)  
(for continuation/divisional with Box 16 completed)
  - i. ☐ DELETION OF INVENTOR(S)  
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b)
  - c. ☐ Unsigned

5. ☐ Microfiche Computer Program (*Appendix*)
6. ☒ Nucleotide and/or Amino Acid Sequence Submission (*if applicable, all necessary*)
  - a. ☒ Computer Readable Copy (from prior application)
  - b. ☒ Paper Copy (identical to computer copy)
  - c. ☒ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. ☐ Assignment Papers (cover sheet & document(s))
8. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney  
(when there is an assignee)
9. ☐ English Translation Document (*if applicable*)
10. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
11. ☒ Preliminary Amendment
12. ☐ Return Receipt Postcard (MPEP 503)  
(Should be specifically itemized)
13. ☒ Small Entity: The Undersigned hereby asserts that Applicants are entitled to small entity status.
14. ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)
15. ☐ Other:

16. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No. 09/081,385, filed May 14, 1998 and of Prior Application No. PCT/US99/10793, filed May 14, 1999.

**UTILITY PATENT APPLICATION TRANSMITTAL**  
 (Only for new non-provisional applications under 37 CFR 1.53(b))

17. CORRESPONDENCE ADDRESS			
Individual Name	Carol L. Francis		
Firm Name	BOZICEVIC, FIELD & FRANCIS LLP		
Address	200 Middlefield Road, Suite 200		
City, State, Zip	Menlo Park, CA 94025		
Country	U.S.A.		
Telephone	(650) 327-3400	Facsimile	(650) 327-3231

SIGNATURE of Applicant or Assignee of Record	
Individual Name	Carol L. Francis
Registration No.	36,513
Signature	<i>Carol L. Francis</i>
Date	<i>November 13, 2000</i>

F:\DOCUMENT\IRVN (UC Irvine)\007con\app transmittal.wpd

# FEE TRANSMITTAL

Note: Effective October 1, 1998.  
Patent fees are subject to annual revision.

Attorney Docket Number	IRVN-007CON
First Named Inventor	Gatanaga, et al.
Application Number	N/A
Filing Date	Herewith
Group Art Unit	N/A
Examiner Name	N/A

## METHOD OF PAYMENT

1. ☐ A Check Is Enclosed
2. ☒ The Commissioner is hereby authorized to charge the following and any additional fees including fees required under 37 CFR 1.16 and 1.17 and credit any overpayments to: Deposit Account No. 50-0815.  
Deposit Account Name: Bozicevic, Field & Francis LLP

## FEE CALCULATION

### 1. FILING FEE

Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	Fee Due
101	710	201	355	Utility filing fee	\$355
102	320	206	160	Design filing fee	
104	490	207	245	Plant filing fee	
109	710	208	355	Reissue filing fee	
110	150	214	75	Provisional filing fee	
Subtotal (1)					\$355

### 2. CLAIMS

<u>No. of claims as filed or after amendment</u>			<u>Most claims previously paid</u>		<u>Extra claims</u>		<u>Fee from below</u>		<u>Fee Due</u>
Total claims	11	-	20	=	00	x	=		\$
Ind. claims	02	-	03	=	00	x	=		
Multiple Dependent claims						x	135	=	\$135

Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description
103	18	203	9	Claims in excess of 20
102	80	202	40	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim
109	80	209	40	Reissue independent claims over original patent
110	18	210	9	Reissue claims in excess of and over original patent
Subtotal (2) \$135				

### 3. ADDITIONAL FEES

Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	Fee Due
105	130	205	65	Surcharge - late filing fee or oath	
139	130	139	130	Non-English specification	
115	110	215	55	Ext. for reply within first month	
117	890	217	445	Ext. for reply within third month	
128	1,890	228	945	Ext. for reply within fifth month	
120	310	220	155	Filing brief in support of appeal	
140	110	240	55	Petition to revive - unavoidable	
142	1,240	242	620	Utility issue fee (or reissue)	
123	50	123	50	Petitions related to prov. appl.	
146	710	246	355	Filing submission after final rejection (37 CFR 1.129(a))	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
143	440	243	220	Design issue fee	
581	40	581	40	Recording patent assignment	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
147	2,520	147	2,520	Filing a request for reexamination	
116	390	216	195	Ext. for reply within second month	
118	1,390	218	695	Ext. for reply within fourth month	
119	310	219	155	Notice of Appeal	
121	270	221	135	Request for oral hearing	
141	1,240	241	620	Petition to revive - unintentional	
122	130	122	130	Petitions to the Commissioner	
126	180	126	180	Submission of IDS	
149	710	249	355	For each additional invention to be examined (37 CFR 1.129(b))	
113	1840*	113	1840*	Requesting publication of SIR after Examiner action	
144	600	244	300	Plant issue fee	
Other fee (specify)					


\* Reduced by Basic Filing Fee Paid

SUBTOTAL (3) \$00.00

**TOTAL AMOUNT TO BE CHARGED (\$)** \$490.00

### SUBMITTED BY

Typed or Printed Name	Carol L. Francis, BOZICEVIC, FIELD & FRANCIS LLP	Reg. Number	36,513
Signature	<i>Carol L. Francis</i>	Deposit Account	50-0815
Date	Nov 13, 2002		

EXPRESS MAIL CERTIFICATE			
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on date indicated above and is addressed to the Commissioner for Patents, Washington, D.C. 20231			
Typed or Printed Name	Mathew Otts	Express Mail No.	EL 563 650 743 US
Signature		Date	November 13, 2000
<b>PRELIMINARY AMENDMENT</b>  Address to: Commissioner for Patents Washington, D.C. 20231		Attorney Docket	IRVN-007CON (UC 96-367-5)
		First Named Inventor	Gatanaga, et al.
		Application Number	N/A
		Filing Date	Herewith
		Group Art Unit	N/A
		Examiner Name	N/A
		Title	Factors Affecting Tumor Necrosis Factor Receptor Releasing Enzyme Activity

Sir:

Prior to the examination on the merits of the above-referenced application, please amend the application as follows.

### AMENDMENTS

#### In the Specification:

On page 1, line 5, after "pending", insert --and of International Application No. PCT/US99/10793, filed May 14, 1999--.

On page 1, line 6, delete "application is" and insert therefore --applications are--.

On page 1, line 6, delete "its" and insert therefore --their--.

#### In The Claims:

Cancel claims 1-36 without prejudice.

Add the following new claims:

-- 37. (New) An isolated polynucleotide comprising a nucleotide sequence expressed at the mRNA level in human mononuclear leukocytes having cell-surface TNF receptor, thereby increasing cleavage and release of the receptor from the surface of the cell.

38. (New) The isolated polynucleotide of claim 1, wherein the nucleotide sequence is expressed at the mRNA level in Jurkat T cells; and when COS-1 cells expressing TNF receptor are genetically altered to express the sequence, the cells have increased enzymatic activity for cleaving and releasing the receptor.

39. (New) The polynucleotide of claim 37 or 38, having one or more of the following properties:

- a) the polynucleotide comprises a nucleotide sequence contained in SEQ ID NOs:1-10;
- b) the polynucleotide comprises a nucleotide sequence of at least 30 consecutive nucleotides contained in SEQ ID NOs:1-10;
- c) the polynucleotide comprises a nucleotide sequence of at least 50 consecutive nucleotides at least 90% identical to a sequence contained in SEQ ID NOs:1-10; or
- d) the polynucleotide is capable of hybridizing specifically to a nucleotide sequence contained in SEQ ID NOs:1-10 under stringent conditions; or
- e) the polynucleotide comprises a nucleotide sequence that encodes at least 10 consecutive amino acids encoded in SEQ ID NOs:1-10;

wherein the polynucleotide has at least one of the following properties:

- i) the polynucleotide encodes a polypeptide which, when incubated with COS-1 cells expressing TNF receptor, promotes enzymatic cleavage and release of the receptor; or
- ii) the polynucleotide is a labeled probe or amplification primer that specifically identifies a polynucleotide comprising SEQ ID NOs:1-10.

40. (New) The polynucleotide of claim 39 that comprises a nucleotide sequence of at least 30 consecutive nucleotides contained in SEQ ID NOs:1-10.

41. (New) The polynucleotide of claim 39 that encodes a polypeptide which, when incubated with COS-1 cells expressing TNF receptor, promotes enzymatic cleavage and release of the receptor

42. (New) The polynucleotide of claim 39 that is a labeled probe or amplification primer.

43. (New) A host cell genetically altered with a polynucleotide according to claim 39.

44. (New) A method for increasing signal transduction from a cytokine into a cell, comprising contacting the cell with a polynucleotide having at least one of the following properties:

- i) the polynucleotide comprises a nucleotide sequence contained in SEQ ID NOs:1-10;
- ii) the polynucleotide comprises a nucleotide sequence of at least 30 consecutive nucleotides contained in SEQ ID NOs:1-10;
- iii) the polynucleotide comprises a nucleotide sequence of at least 50 consecutive nucleotides at least 90% identical to a sequence contained in SEQ ID NOs:1-10; or
- iv) the polynucleotide is capable of hybridizing specifically to a nucleotide sequence contained in SEQ ID NOs:1-10 under stringent conditions.

45. (New) The method of claim 44, wherein the polynucleotide comprises a nucleotide sequence of at least 30 consecutive nucleotides contained in SEQ ID NOs:1-10.

46. (New) The method of claim 44, wherein the cytokine is TNF.--

#### REMARKS

Claims 37-46 are pending after entry of the amendments above.

Claims 1-36 are canceled without prejudice to renewal, without intent to abandon any subject matter therein, and without acquiescing to any rejection which may have been applied. Applicants expressly reserve the right to pursue the subject matter of the canceled claims in a continuing application.

Support for new claims 37-38 is found in, for example, claim 1 as originally filed.

Support for new claims 39-40 is found in, for example, claims 2-5 and 9 as originally filed.

Support for new claim 41 is found in, for example, claim 11 as originally filed.

Support for new claims 42 is found in, for example, the specification at page 11, line 14 and page 17, lines 1-11.

Support for new claim 43 is found in, for example, the specification at page 14, lines 26-27.

Support for new claim 44 is found in, for example, claim 22 as originally filed.

Support for new claim 46 is found in, for example, claim 23 as originally filed.

No new matter is added.

### CONCLUSION

Applicants respectfully submit that the claims are in form for allowance, early notice of which is requested. If, in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 327-3400.

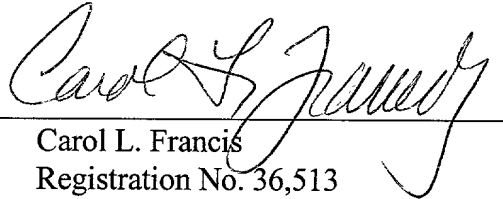
The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815.

Respectfully submitted,  
BOZICEVIC, FIELD & FRANCIS LLP

Date:

November 13, 2000

By:


  
Carol L. Francis  
Registration No. 36,513

BOZICEVIC, FIELD & FRANCIS LLP  
200 Middlefield Road, Suite 200  
Menlo Park, CA 94025  
Telephone: (650) 327-3400  
Facsimile: (650) 327-3231

F:\DOCUMENT\IRVN (UC IRVINE)\007CON\PRELIMINARY AMENDMENT IRVN-007CON.DOC

Atty Docket No.: IRVN-007CON

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
Signature of Person Mailing Paper or Fee

for

[illegible]

F:\MJO\Docs\Pats\covers\Application Cover Page.frm



**FACTORS AFFECTING TUMOR NECROSIS FACTOR RECEPTOR  
RELEASING ENZYME ACTIVITY**

**CROSS-REFERENCE TO RELATED APPLICATIONS**

5 This application claims the priority benefit of U.S. application 09/081,385,  
filed May 14, 1998, pending. For purposes of prosecution in the U.S., the priority  
application is hereby incorporated herein by reference in its entirety.

**FIELD OF THE INVENTION**

10 This invention relates generally to the field of signal transduction between  
cells, via cytokines and their receptors. More specifically, it relates to enzymatic  
activity that cleaves and releases the receptor for TNF found on the cell surface,  
and the consequent biological effects. Certain embodiments of this invention are  
compositions that affect such enzymatic activity, and may be included in  
medicaments for disease treatment.

15

**BACKGROUND OF THE INVENTION**

20 Cytokines play a central role in the communication between cells.  
Secretion of a cytokine from one cell in response to a stimulus can trigger an  
adjacent cell to undergo an appropriate biological response — such as  
stimulation, differentiation, or apoptosis. It is hypothesized that important  
biological events can be influenced not only by affecting cytokine release from  
the first cell, but also by binding to receptors on the second cell, which mediates  
the subsequent response. The invention described in this patent application  
provides new compounds for affecting signal transduction from tumor necrosis  
25 factor.

The cytokine known as tumor necrosis factor (TNF or TNF- $\alpha$ ) is  
structurally related to lymphotoxin (LT or TNF- $\beta$ ). They have about 40 percent  
amino acid sequence homology (Old, *Nature* 330:602-603, 1987). These  
cytokines are released by macrophages, monocytes and natural killer cells and

play a role in inflammatory and immunological events. The two cytokines cause a broad spectrum of effects both in vitro and in vivo, including: (i) vascular thrombosis and tumor necrosis; (ii) inflammation; (iii) activation of macrophages and neutrophils; (iv) leukocytosis; (v) apoptosis; and (vi) shock. TNF has been associated with a variety of disease states including various forms of cancer, arthritis, psoriasis, endotoxic shock, sepsis, autoimmune diseases, infections, obesity, and cachexia. TNF appears to play a role in the three factors contributing to body weight control: intake, expenditure, and storage of energy (Rothwell, *Int. J. Obesity* 17:S98-S101, 1993). In septicemia, increased endotoxin concentrations appear to raise TNF levels (Beutler et al. *Science* 229:869-871, 1985).

Attempts have been made to alter the course of a disease by treating the patient with TNF inhibitors, with varying degrees of success. For example, the TNF inhibitor dexamabiol provided protection against TNF mediated effects following traumatic brain injury (Shohami et al. *J. Neuroimmun.* 72:169-77, 1997). Some improvement in Crohn's disease was afforded by treatment with anti-TNF antibodies (Neurath et al., *Eur. J. Immun.* 27:1743-50, 1997).

Human TNF and LT mediate their biological activities by binding specifically to two distinct glycoprotein plasma membrane receptors (55 kDa and 75 kDa in size, known as p55 and p75 TNF-R, respectively). The two receptors share 28 percent amino acid sequence homology in their extracellular domains, which are composed of four repeating cysteine-rich regions (Tartaglia and Goeddel, *Immunol. Today* 13:151-153, 1992). However, the receptors lack significant sequence homology in their intracellular domains, and mediate different intracellular responses to receptor activation. In accordance with the different activities of TNF and LT, most human cells express low levels of both TNF receptors: about 2,000 to 10,000 receptors per cell (Brockhaus et al., *Proc. Natl. Acad. Sci. USA* 87:3127-3131, 1990).

Expression of TNF receptors on both lymphoid and non-lymphoid cells can be influenced experimentally by many different agents, such as bacterial lipopolysaccharide (LPS), phorbol myristate acetate (PMA; a protein kinase C

activator), interleukin-1 (IL-1), interferon-gamma (IFN- $\gamma$ ) and IL-2 (Gatanaga et al. *Cell Immunol.* 138:1-10, 1991; Yui et al. *Placenta* 15:819-835, 1994). It has been shown that complexes of human TNF bound to its receptor are internalized from the cell membrane, and then the receptor is either degraded or recycled (Armitage, *Curr. Opin. Immunol.* 6:407-413, 1994). It has been proposed that TNF receptor activity can be modulated using peptides that bind intracellularly to the receptor, or which bind to the ligand binding site, or that affect receptor shedding. See for example patent publications WO 95/31544, WO 95/33051, WO 96/01642, and EP 568 925.

TNF binding proteins (TNF-BP) have been identified at elevated levels in the serum and urine of febrile patients, patients with renal failure, and cancer patients, and even certain healthy individuals. Human brain and ovarian tumors produced high serum levels of TNF-BP. These molecules have been purified, characterized, and cloned (Gatanaga et al., *Lymphokine Res.* 9:225-229, 1990a; Gatanaga et al., *Proc. Natl. Acad. Sci USA* 87:8781-8784, 1990b). Human TNF-BP consists of 30 kDa and 40 kDa proteins which are identical to the N-terminal extracellular domains of p55 and p75 TNF receptors, respectively (US Patent No. 5,395,760; EP 418,014). Such proteins have been suggested for use in treating endotoxic shock. Mohler et al. *J. Immunol.* 151:1548-1561, 1993

There are several mechanisms possible for the production of secreted proteins resembling membrane bound receptors. One involves translation from alternatively spliced mRNAs lacking transmembrane and cytoplasmic regions. Another involves proteolytic cleavage of the intact membrane receptors, followed by shedding of the cleaved receptor from the cell. The soluble form of p55 and p75 TNF-R do not appear to be generated from mRNA splicing, since only full length receptor mRNA has been detected in human cells *in vitro* (Gatanaga et al., 1991). Carboxyl-terminal sequencing and mutation studies on human p55 TNF-R indicates that a cleavage site may exist between residues Asn 172 and Val 173 (Gullberg et al. *Eur. J. Cell. Biol.* 58:307-312, 1992).

There are reports that a specific metalloprotease inhibitor, TNF- $\alpha$  protease inhibitor (TAPI) blocks the shedding of soluble p75 and p55 TNF-R (Crowe et al.



modulated, thereby controlling the signal transduction from TNF at a disease site.

### SUMMARY OF THE INVENTION

5        This disclosure provides new compounds that promote enzymatic cleavage and release of TNF receptors from the cell surface. Nine new DNA clones have been selected after repeat screening in an assay that tests the ability to enhance receptor release. The polynucleotide sequences of this invention and the proteins encoded by them have potential as diagnostic aids,  
10       and therapeutic compounds that can be used to adjust TNF signal transduction in a beneficial way.

One embodiment of the invention is an isolated polynucleotide comprising a nucleotide sequence with the following properties: a) the sequence is expressed at the mRNA level in Jurkat T cells; b) when COS-1 cells expressing  
15       TNF-receptor are genetically transformed to express the sequence, the cells have increased enzymatic activity for cleaving and releasing the receptor. If a polynucleotide sequence is expressed in Jurkat cells, then it can be found in the Jurkat cell expression library deposited with the ATCC (Accession No. TIB-152). It is recognized that the polynucleotide can be obtained from other cell lines, or  
20       produced by recombinant techniques.

Included are polynucleotides in which the nucleotide sequence is contained in any of SEQ. ID NOS:1-10. Also embodied are polynucleotides comprising at least 30 and preferably more consecutive nucleotides in said nucleotide sequence, or at least 50 consecutive nucleotides that are homologous  
25       to said sequence at a significant level, preferably at the 90% level or more. Also included antisense and ribozyme polynucleotides that inhibit the expression of a TRRE modulator.

Another embodiment of the invention is isolated polypeptides comprising an amino acid sequence encoded by a polynucleotide of this invention. Non-  
30       limiting examples are sequences shown in SEQ. ID NOS: 147-158. Fragments

and fusion proteins are included in this invention, and preferably comprise at least 10 consecutive residues encoded by a polynucleotide of this invention, or at least 15 consecutive amino acids that are homologous at a significant level, preferably at least 80%. Preferred polypeptides promote cleavage and release  
5 of TNF receptors from the cell surface, especially COS-1 cells genetically transformed to express TNF receptor. The polypeptides may or may not have a membrane spanning domain, and may optionally be produced by a process that involves secretion from a cell. Included are species homologs with the desired activity, and artificial mutants with additional beneficial properties.

10 Another embodiment of this invention is an antibody specific for a polypeptide of this invention. Preferred are antibodies that bind a TRRE modulator protein, but not other substances found in human tissue samples in comparable amounts.

Another embodiment of the invention is an assay method of determining  
15 altered TRRE activity in a cell or tissue sample, using a polynucleotide or antibody of this invention to detect the presence or absence of the corresponding TRRE modulator. The assay method can optionally be used for the diagnosis or evaluation of a clinical condition relating to abnormal TNF levels or TNF signal transduction.

20 Another embodiment of the invention is a method for increasing or decreasing signal transduction from a cytokine into a cell (including but not limited to TNF), comprising contacting the cell with a polynucleotide, polypeptide, or antibody of this invention.

A further embodiment of the invention is a method for screening  
25 polynucleotides for an ability to modulate TRRE activity. The method involves providing cells that express both TRRE and the TNF-receptor; genetically altering the cells with the polynucleotides to be screened; cloning the cells; and identifying clones with the desired activity.

Yet another embodiment of the invention is a method for screening  
30 substances for an ability to affect TRRE activity. This typically involves incubating cells expressing TNF receptor with a TRRE modulator of this

invention in the presence or absence of the test substance; and measuring the effect on shedding of the TNF receptor .

The products of this invention can be used in the preparation of a medicament for treatment of the human or animal body. The medicament contains a clinically effective amount for treatment of a disease such as heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, sepsis, and cancer. These compositions can be used for administration to a subject suspected of having or being at risk for the disease, optionally in combination with other forms of treatment appropriate for their condition.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** is a schematic representation of plasmid pCDTR2. This plasmid expresses p75 TNF-R, the ~75 kDa form of the TNF receptor. PCMV stands for cytomegalovirus; BGHpA stands for bovine growth hormone polyadenylation signal.

**Figure 2** is a line depicting the levels of p75 TNF-R detected on COS-1 cells genetically altered to express the receptor. Results from the transformed cells, designated C75R (●, upward swooping line) is compared with that from the parental COS-1 cells (■, baseline). The receptor number was calculated by Scatchard analysis (inset).

**Figure 3** is a survival graph, showing that TRRE decreases mortality in mice challenged with lipopolysaccharide (LPS) to induce septic peritonitis. (♦) LPS alone; (■) LPS plus control buffer; (●) LPS plus TRRE (2,000 U); (▲) LPS plus TRRE (4,000 U).

**Figure 4** is a half-tone reproduction of a bar graph, showing the effect of 9 new clones on TRRE activity on C75R cells (COS-1 cells transfected to express the TNF-receptor. Each of the 9 clones increases TRRE activity by over 2-fold.

**Figure 5** is a survival graph, showing the ability of 4 new expressed to save mice challenged with LPS. (♦) saline; (■) BSA; (Δ) Mey-3 (100 μg); (X) Mey-3 (10 μg); (\*) Mey-5 (10 μg); (●) Mey-8 (10 μg).

## DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that certain cells involved in the TNF transduction pathway express enzymatic activity that causes TNF receptors to be shed from the cell surface. Enzymatic activity for cleaving and releasing TNF receptors has been given the designation TRRE. Phorbol myristate acetate induces release of TRRE from cells into the culture medium. An exemplary TRRE protein had been purified from the supernatant of TNF-1 cells (Example 2). The protease bears certain hallmarks of the metalloprotease family, and is released rapidly from the cell upon activation.

In order to elucidate the nature of this protein, functional cloning was performed. Jurkat cells were selected as being a good source of TRRE. The cDNA from a Jurkat library was expressed, and cell supernatant was tested for an ability to release TNF receptors from cell surfaces. Cloning and testing of the expression product was conducted through several cycles, and nine clones were obtained that more than doubled TRRE activity in the assay (Figure 4). At the DNA level, all 9 clones had different sequences.

Protein expression products from the clones have been tested in a lipopolysaccharide animal model for sepsis. Protein from three different clones successfully rescued animals from a lethal dose of LPS (Figure 5). This points to an important role for these molecules in the management of pathological conditions mediated by TNF.



The number of new TRRE promoting clones obtained from the expression library was surprising. The substrate specificity of the TRRE isolated in Example 2 distinguishes the 75 kDa and 55 kDa TNF receptors from other cytokine receptors and cell surface proteins. There was little reason beforehand to suspect that cells might have nine different proteases for the TNF receptor. It is possible that one of the clones encodes the TRRE isolated in Example 2, or a related protein. It is possible that some of the other clones have proteolytic activity to cleave TNF receptors at the same site, or at another site that causes release of the soluble form from the cell. It is a hypothesis of this disclosure that some of the clones may not have proteolytic activity themselves, but play a role in promoting TRRE activity in a secondary fashion.

This possibility is consistent with the observations made, because there is an endogenous level of TRRE activity in the cells used in the assay. The cleavage assay involves monitoring TNF receptor release from C75 cells, which are COS-1 cells genetically altered to express p75 TNF-R. The standard assay is conducted by contacting the transformed cells with a fluid believed to contain TRRE. The level of endogenous TRRE activity is evident from the rate of spontaneous release of the receptor even when no exogenous TRRE is added (about 200 units). Accordingly, accessory proteins that promote TRRE activity would increase the activity measured in the assay. Many mechanisms of promotion are possible, including proteins that activate a zymogen form of TRRE, proteins that free TRRE from other cell surface components, or proteins that stimulate secretion of TRRE from inside the cell. It is not necessary to understand the mechanism in order to use the products of this invention in most of the embodiments described.

It is anticipated that several of the clones will have activity not just for promoting TNF receptor cleavage, but also having an effect on other surface proteins. To the extent that cleavage sequences or accessory proteins are shared between different receptors, certain clones would promote phenotypic change (such as receptor release) for the family of related substrates.

This disclosure provides polypeptides that promote TRRE activity, polynucleotides that encode such polypeptides, and antibodies that bind such peptides. The binding of TNF to its receptor mediates a number of biological effects. Cleavage of the TNF-receptor by TRRE diminishes signal transduction  
 5 by TRRE. Potentiators of TRRE activity have the same effect. Thus, the products of this invention can be used to modulate signal transduction by cytokines, which is of considerable importance in the management of disease conditions that are affected by cytokine action. The products of this invention can also be used in diagnostic methods, to determine when signal transduction is  
 10 being inappropriately affected by abnormal TRRE activity. The assay systems described in this disclosure provide a method for screening additional compounds that can influence TRRE activity, and thus the signal transduction from TNF.

Based on the summary of the invention, and guided by the illustrations in  
 15 the example section, one skilled in the art will readily know what techniques to employ in the practice of the invention. The following detailed description is provided for the additional convenience of the reader.

#### Definitions and basic techniques

20 As used in this disclosure, "TRRE activity" refers to the ability of a composition to cleave and release TNF receptors from the surface of cells expressing them. A preferred assay is cleavage from transfected COS-1 cells, as described in Example 1. However, TRRE activity can be measured on any cells that bear TNF receptors of the 55 kDa or 75 kDa size. Other features of the  
 25 TRRE enzyme obtained from PMA induction of THP-1 cells (exemplified in Example 2) need not be a property of the TRRE activity measured in the assay.

Unit activity of TRRE is defined as 1 pg of soluble p75 TNF-R released from cell surface in a standard assay, after correction for spontaneous release. The measurement of TRRE activity is explained further in Example 1.

30 A "TRRE modulator" is a compound that has the property of either increasing or decreasing TRRE activity for processing TNF on the surface of

cells. Those that increase TRRE activity may be referred to as TRRE promoters, and those that decrease TRRE activity may be referred to as TRRE inhibitors. TRRE promoters include compounds that have proteolytic activity for TNF-R, and compounds that augment the activity of TNF-R proteases. The nine  
5 polynucleotide clones described in Example 5, and their protein products, are exemplary TRRE promoters. Inhibitors of TRRE activity can be obtained using the screening assays described below.

The term "polynucleotide" refers to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof.  
10 Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, (mRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, nucleic acid probes, and primers. A polynucleotide may comprise  
15 modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide refers interchangeably to double-and single-stranded molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide  
20 encompasses both the double-stranded form, and each of two complementary single-stranded forms known or predicted to make up the double-stranded form

"Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. Hybridization reactions can be performed  
25 under conditions of different "stringency". Relevant conditions include temperature, ionic strength, and the presence of additional solutes in the reaction mixture such as formamide. Conditions of increasing stringency are 30°C. in 10X SSC (0.15M NaCl, 15 mM citrate buffer); 40°C. in 6X SSC; 50°C. in 6X SSC 60°C. in 6X SSC, or at about 40°C. in 0.5X SSC, or at about 30°C. in 6X.  
30 SSC containing 50% formamide. SDS and a source of fragmented DNA (such as salmon sperm) are typically also present during hybridization. Higher

stringency requires higher minimum complementarity between hybridizing elements for a stable hybridization complex to form. See "Molecular Cloning: A Laboratory Manual", Second Edition (Sambrook, Fritsch & Maniatis, 1989).

It is understood that purine and pyrimidine nitrogenous bases with similar structures can be functionally equivalent in terms of Watson-Crick base-pairing; and the inter-substitution of like nitrogenous bases, particularly uracil and thymine, or the modification of nitrogenous bases, such as by methylation, does not constitute a material substitution.

The percentage of sequence identity for polynucleotides or polypeptides is calculated by aligning the sequences being compared, and then counting the number of shared residues at each aligned position. No penalty is imposed for the presence of insertions or deletions, but are permitted only where required to accommodate an obviously increased number of amino acid residues in one of the sequences being aligned. When one of the sequences being compared is indicated as being "consecutive", then no gaps are permitted in that sequence during the comparison. The percentage identity is given in terms of residues in the test sequence that are identical to residues in the comparison or reference sequence.

As used herein, "expression" of a polynucleotide refers to the production of an RNA transcript. Subsequent translation into protein or other effector compounds may also occur, but is not required unless specified.

"Genetic alteration" refers to a process wherein a genetic element is introduced into a cell other than by mitosis or meiosis. The element may be heterologous to the cell, or it may be an additional copy or improved version of an element already present in the cell. Genetic alteration may be effected, for example, by transducing a cell with a recombinant plasmid or other polynucleotide through any process known in the art, such as electroporation, calcium phosphate precipitation, or contacting with a polynucleotide-liposome complex. Genetic alteration may also be effected, for example, by transduction or infection with a DNA or RNA virus or viral vector. It is preferable that the

genetic alteration is inheritable by progeny of the cell, but this is not generally required unless specified.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component.

A "fusion polypeptide" is a polypeptide comprising regions in a different position in the sequence than occurs in nature. The regions can normally exist in separate proteins and are brought together in the fusion polypeptide; they can normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide; or they can be synthetically arranged. A "functionally equivalent fragment" of a polypeptide varies from the native sequence by addition, deletion, or substitution of amino acid residues, or any combination thereof, while preserving a functional property of the fragment relevant to the context in which it is being used. Fusion peptides and functionally equivalent fragments are included in the definition of polypeptides used in this disclosure.

It is understood that the folding and the biological function of proteins can accommodate insertions, deletions, and substitutions in the amino acid sequence. Some amino acid substitutions are more easily tolerated. For example, substitution of an amino acid with hydrophobic side chains, aromatic side chains, polar side chains, side chains with a positive or negative charge, or side chains comprising two or fewer carbon atoms, by another amino acid with a side chain of like properties can occur without disturbing the essential identity of the two sequences. Methods for determining homologous regions and scoring the degree of homology are described in Altschul et al. *Bull. Math. Bio.* 48:603-616, 1986; and Henikoff et al. *Proc. Natl. Acad. Sci. USA* 89:10915-10919, 1992. Substitutions that preserve the functionality of the polypeptide, or confer a new

and beneficial property (such as enhanced activity, stability, or decreased immunogenicity) are especially preferred.

An "antibody" (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a polypeptide, through  
5 at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact antibodies, but also antibody equivalents that include at least one antigen combining site of the desired specificity. These include but are not limited to enzymatic or recombinantly produced fragments antibody, fusion proteins,  
10 humanized antibodies, single chain variable regions, diabodies, and antibody chains that undergo antigen-induced assembly.

An "isolated" polynucleotide, polypeptide, protein, antibody, or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar  
15 substance naturally occurs or is initially obtained from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing  
20 enrichments of the embodiments of this invention are increasingly more preferred. Thus, for example, a 2-fold enrichment is preferred, 10-fold enrichment is more preferred, 100-fold enrichment is more preferred, 1000-fold enrichment is even more preferred. A substance can also be provided in an isolated state by a process of artificial assembly, such as by chemical synthesis  
25 or recombinant expression.

A "host cell" is a cell which has been genetically altered, or is capable of being transformed, by administration of an exogenous polynucleotide.

The term "clinical sample" encompasses a variety of sample types obtained from a subject and useful in an in vitro procedure, such as a diagnostic  
30 test. The definition encompasses solid tissue samples obtained as a surgical removal, a pathology specimen, or a biopsy specimen, cells obtained from a

clinical subject or their progeny obtained from culture, liquid samples such as blood, serum, plasma, spinal fluid, and urine, and any fractions or extracts of such samples that contain a potential indication of the disease.

Unless otherwise indicated, the practice of the invention will employ  
5 conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, within the skill of the art. Such techniques are explained in the standard literature, such as: "Molecular Cloning: A Laboratory Manual", Second Edition (Sambrook, Fritsch & Maniatis, 1989), "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984), "Animal Cell Culture" (R. I. Freshney, ed., 1987); the series  
10 "Methods in Enzymology" (Academic Press, Inc.); "Handbook of Experimental Immunology" (D. M. Weir & C. C. Blackwell, Eds.), "Gene Transfer Vectors for Mammalian Cells" (J. M. Miller & M. P. Calos, eds., 1987), "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987); and "Current Protocols in Immunology" (J. E. Coligan et al., eds., 1991). The reader may also choose to  
15 refer to a previous patent application relating to TRRE, International Patent Application WO 98020140.

For purposes of prosecution in the U.S., and in other jurisdictions where  
allowed, all patents, patent applications, articles and publications indicated  
anywhere in this disclosure are hereby incorporated herein by reference in their  
20 entirety.

### Polynucleotides

Polynucleotides of this invention can be prepared by any suitable  
technique in the art. Using the data provided in this disclosure, sequences of  
25 less than ~50 base pairs are conveniently prepared by chemical synthesis, either through a commercial service or by a known synthetic method, such as the triester method or the phosphite method. A preferred method is solid phase synthesis using mononucleoside phosphoramidite coupling units (Hirose et al.,  
*Tetra. Lett.* 19:2449-2452, 1978; U.S. Patent No. 4,415,732).

30 For use in antisense therapy, polynucleotides can be prepared by chemistry that produce more stable in pharmaceutical preparations. Non-limiting

examples include thiol-derivatized nucleosides (U.S. Patent 5,578,718), and oligonucleotides with modified backbones (U.S. Patent Nos. 5,541,307 and 5,378,825).

Polynucleotides of this invention can also be obtained by PCR  
5 amplification of a template with the desired sequence. Oligonucleotide primers spanning the desired sequence are annealed to the template, elongated by a DNA polymerase, and then melted at higher temperature so that the template and elongated oligonucleotides dissociate. The cycle is repeated until the desired amount of amplified polynucleotide is obtained (U.S. Patent Nos.  
10 4,683,195 and 4,683,202). Suitable templates include the Jurkat T cell library and other human or animal expression libraries that contain TRRE modulator encoding sequences. The Jurkat T cell library is available from the American Type Culture Collection, 10801 University Blvd., Manassas VA 20110, U.S.A. (ATCC #TIB-152). Mutations and other adaptations can be performed during  
15 amplification by designing suitable primers, or can be incorporated afterwards by genetic splicing.

Production scale amounts of large polynucleotides are most conveniently obtained by inserting the desired sequence into a suitable cloning vector and reproducing the clone. Techniques for nucleotide cloning are given in Sambrook,  
20 Fritsch & Maniatis (supra) and in U.S. Patent No. 5,552,524. Exemplary cloning and expression methods are illustrated in Example 6.

Preferred polynucleotide sequences are 50%, 70%, 80% , 90%, or 100% identical to one of the sequences exemplified in this disclosure; in order of increasing preference. The length of consecutive residues in the identical or  
25 homologous sequence compared with the exemplary sequence can be about 15, 30, 50, 75, 100, 200 or 500 residues in order of increasing preference, up to the length of the entire clone. Nucleotide changes that cause a conservative substitution or retain the function of the encoded polypeptide (in terms of hybridization properties or what is encoded) are especially preferred  
30 substitutions.



Abstract

The polynucleotides of this can be used to measure altered TRRE activity in a cell or tissue sample. This involves contacting the sample with the polynucleotide under conditions that permit the polynucleotide to hybridize specifically with nucleic acid that encodes a modulator of TRRE activity, if present in the sample, and determining polynucleotide that has hybridized as a result of step a). Specificity of the test can be provided in one of several ways. One method involves the use of a specific probe — a polynucleotide of this invention with a sequence long enough and of sufficient identity to the sequence being detected, so that it binds the target and not other nucleic acid that might be present in the sample. The probe is typically labeled (either directly or through a secondary reagent) so that it can be subsequently detected. Suitable labels include  $^{32}\text{P}$  and  $^{33}\text{P}$ , chemiluminescent and fluorescent reagents. After the hybridization reaction, unreacted probe is washed away so that the amount of hybridized probe can be determined. Signal can be amplified using branched probes (U.S. Patent No. 5,124,246). In another method, the polynucleotide is a primer for a PCR reaction. Specificity is provided by the ability of the paired probes to amplify the sequence of interest. After a suitable number of PCR cycles, the amount of amplification product present correlates with the amount of target sequence originally present in the sample.

Such tests are useful both in research, and in the diagnosis or assessment of a disease condition. For example, TNF activity plays a role in eliminating tumor cells (Example 4), and a cancer may evade the elimination process by activating TRRE activity in the diseased tissue. Hence, under some conditions, high expression of TRRE modulators may correlate with progression of cancer. Diagnostic tests are also of use in monitoring therapy, such as when gene therapy is performed to increase TRRE activity.

Polynucleotides of this invention can also be used for production of polypeptides and the preparation of medicaments, as explained below.

## Polypeptides

Short polypeptides of this invention can be prepared by solid-phase chemical synthesis. The principles of solid phase chemical synthesis can be found in Dugas & Penney, Bioorganic Chemistry, Springer-Verlag NY pp 54-92 (1981), and U.S. Patent No. 4,493,795. Automated solid-phase peptide synthesis can be performed using devices such as a PE-Applied Biosystems 430A peptide synthesizer (commercially available from Applied Biosystems, Foster City CA).

Longer polypeptides are conveniently obtained by expression cloning. A polynucleotide encoding the desired polypeptide is operably linked to control elements for transcription and translation, and then transfected into a suitable host cell. Expression may be effected in procaryotes such as *E. coli* (ATCC Accession No. 31446 or 27325), eukaryotic microorganisms such as the yeast *Saccharomyces cerevisiae*, or higher eukaryotes, such as insect or mammalian cells. A number of expression systems are described in U.S. Patent No. 5,552,524. Expression cloning is available from such commercial services as Lark Technologies, Houston TX. The production of protein from 4 exemplary clones of this invention in insect cells is illustrated in Example 6. The protein is purified from the producing host cell by standard methods in protein chemistry, such as affinity chromatography and HPLC. Expression products are optionally produced with a sequence tag to facilitate affinity purification, which can subsequently be removed.

Preferred sequences are 40%, 60%, 80% , 90%, or 100% identical to one of the sequences exemplified in this disclosure; in order of increasing preference. The length of the identical or homologous sequence compared with the native human polynucleotide can be about 7, 10, 15, 20, 30, 50 or 100 residues in order of increasing preference, up to the length of the entire encoding region.

Polypeptides can be tested for an ability to modulate TRRE in a TNF-R cleavage assay. The polypeptide is contacted with the receptor (preferably expressed on the surface of a cell, such as a C75 cell), and the ability of the polypeptide to increase or decrease receptor cleavage and release is

determined. Cleavage of TNF-R by exemplary polypeptides of this invention is illustrated in Example 7.

Polypeptides of this invention can be used as immunogens for raising antibody. Large proteins will raise a cocktail of antibodies, while short peptide fragments will raise antibodies against small region of the intact protein. Antibody clones can be mapped for protein binding site by producing short overlapping peptides of about 10 amino acids in length. Overlapping peptides can be prepared on a nylon membrane support by standard F-Moc chemistry, using a SPOTS™ kit from Genosys according to manufacturer's directions.

Polypeptides of this invention can also be used to affect TNF signal transduction, as explained below.

#### Antibodies

Polyclonal antibodies can be prepared by injecting a vertebrate with a polypeptide of this invention in an immunogenic form. Immunogenicity of a polypeptide can be enhanced by linking to a carrier such as KLH, or combining with an adjuvant, such as Freund's adjuvant. Typically, a priming injection is followed by a booster injection is after about 4 weeks, and antiserum is harvested a week later. Unwanted activity cross-reacting with other antigens, if present, can be removed, for example, by running the preparation over adsorbants made of those antigens attached to a solid phase, and collecting the unbound fraction. If desired, the specific antibody activity can be further purified by a combination of techniques, which may include protein, A chromatography, ammonium sulfate precipitation, ion exchange chromatography, HPLC, and immunoaffinity chromatography using the immunizing polypeptide coupled to a solid support. Antibody fragments and other derivatives can be prepared by standard immunochemical methods, such as subjecting the antibody to cleavage with enzymes such as papain or pepsin.

Production of monoclonal antibodies is described in such standard references as Harrow & Lane (1988), U.S. Patent Nos. 4,491,632, 4,472,500 and 4,444,887, and *Methods in Enzymology* 73B:3 (1981). Briefly, a mammal is

immunized, and antibody-producing cells (usually splenocytes) are harvested. Cells are immortalized by fusion with a non-producing myeloma, transfecting with Epstein Barr Virus, or transforming with oncogenic DNA. The treated cells are cloned and cultured, and the clones are selected that produce antibody of the  
5 desired specificity.

Other methods of obtaining specific antibody molecules (optimally in the form of single-chain variable regions) involve contacting a library of immunocompetent cells or viral particles with the target antigen, and growing out positively selected clones. Immunocompetent phage can be constructed to  
10 express immunoglobulin variable region segments on their surface. See Marks et al., *New Eng. J. Med.* 335:730, 1996, International Patent Applications WO 9413804, WO 9201047, WO 90 02809, and McGuiness et al., *Nature Biotechnol.* 14:1449, 1996.

The antibodies of this invention are can be used in immunoassays for  
15 TRRE modulators. General techniques of immunoassay can be found in "The Immunoassay Handbook", Stockton Press NY, 1994; and "Methods of Immunological Analysis", Weinheim: VCH Verlags gesellschaft mbH, 1993). The antibody is combined with a test sample under conditions where the antibody will bind specifically to any modulator that might be present, but not any other  
20 proteins liable to be in the sample. The complex formed can be measured in situ (U.S. Patent Nos. 4,208,479 and 4,708,929), or by physically separating it from unreacted reagents (U.S. Patent No. 3,646,346). Separation assays typically involve labeled TRRE reagent (competition assay), or labeled antibody (sandwich assay) to facilitate detection and quantitation of the complex. Suitable  
25 labels are radioisotopes such as <sup>125</sup>I, enzymes such as  $\beta$ -galactosidase, and fluorescent labels such as fluorescein. Antibodies of this invention can also be used to detect TRRE modulators in fixed tissue sections by immunohistology. The antibody is contacted with the tissue, unreacted antibody is washed away, and then bound antibody is detected — typically using a labeled anti-  
30 immunoglobulin reagent. Immunohistology will show not only whether the modulator is present, but where it is located in the tissue.

Detection of TRRE modulators is of interest for research purposes, and for clinical use. As indicated earlier, high expression of TRRE modulators may correlate with progression of cancer. Diagnostic tests are also of use in monitoring TRRE modulators that are administered in the course of therapy.

Antibodies of this invention can also be used for preparation of medicaments. Antibodies with therapeutic potential include those that affect TRRE activity — either by promoting clearance of a TRRE modulator, or by blocking its physiological action. Antibodies can be screened for desirable activity according to assays described in the next section.

### Screening assays

This invention provides a number of screening methods for selecting and developing products that modulate TRRE, and thus affect TNF signal transduction.

One screening method is for polynucleotides that have an ability to modulate TRRE activity. To do this screening, cells are obtained that express both TRRE and the TNF receptor. Suitable cell lines can be constructed from any cell that expresses a level of functional TRRE activity. These cells are identifiable by testing culture supernatant for an ability to release membrane-bound TNF-R. The level of TRRE expression should be moderate, so that an increase in activity can be detected. The cells can then be genetically altered to express either p55 or p75 TNF-R, illustrated in Example 1. Exemplary is the C75R line: COS-1 cells genetically altered to express the 75 kDa form of the TNF-R. Release of TNF-R from the cell can be measured either by testing residual binding of labeled TNF ligand to the cell, or by immunoassay of the supernatant for released receptor (Example 1).

The screening assay is conducted by contacting the cells expressing TRRE and TNF-R with the polynucleotides to be screened. The effect of the polynucleotide on the enzymatic release of TNF-R from the cell is determined, and polynucleotides with desirable activity (either promoting or inhibiting TRRE activity) are selected. In a variation of this method, cells expressing TRRE

activity but not TNF-R (such as untransfected COS-1 cells) are contacted with the test polynucleotide. Then the culture medium is collected, and used to assay for TRRE activity using a second cell expressing TNF-R (such as C75 cells).

This type of screening assay is useful for the selection of polynucleotides from an expression library believed to contain encoding sequences for TRRE modulators. The Jurkat cell expression library (ATCC Accession No. TIB-152) is exemplary. Other cells from which suitable libraries can be constructed are those known to express high levels of TRRE, especially after PMA stimulation, such as THP-1, U-937, HL-60, ME-180, MRC-5, Raji, K-562, and normal human monocytes. The screening involves expressing DNA from the library in the selected cell line being used for screening. Wells with the desired activity are selected, and the DNA is recovered, optionally after replication or cloning of the cells. Repeat cycles of functional screening and selection can lead to identification of new polynucleotide clones that promote or inhibit TRRE activity. This is illustrated below in Example 5. Further experiments can be performed on the selected polynucleotides to determine if it modulates TRRE activity inside the cell, or through the action of a protein product. A long open reading frame suggests a role for a protein product, and examination of the amino acid sequence for a signal peptide and a membrane spanning region can help determine whether the protein is secreted from the cell or expressed in the surface membrane.

This type of screening is also useful for further development of the polynucleotides of this invention. For example, expression constructs can be developed that encode functional peptide fragments, fusion proteins, and other variants. The minimum size of polynucleotide sequence that still encodes TRRE modulation activity can be determined by removing part of the sequence and then using the screening assay to determine whether the activity is still present. Mutated and extended sequences can be tested in the same way.

This type of screening assay is also useful for developing compounds that affect TRRE activity by interfering with mRNA that encode a TRRE modulator. Of particular interest are ribozymes and antisense oligonucleotides. Ribozymes

are endoribonucleases that catalyze cleavage of RNA at a specific site. They comprise a polynucleotide sequence that is complementary to the cleavage site on the target, and additional sequence that provide the tertiary structure to effect the cleavage. Construction of ribozymes is described in U.S. Patent Nos. 4,987,071 and 5,591,610. Antisense oligonucleotides that bind mRNA comprise a short sequence complementary to the mRNA (typically 8-25 bases in length). Preferred chemistry for constructing antisense oligonucleotides is outlined in an earlier section. Specificity is provided both by the complementary sequence, and by features of the chemical structure. Antisense molecules that inhibit expression of cell surface receptors are described in U.S. Patent Nos. 5,135,917 and 5,789,573. Screening involves contacting the cell expressing TRRE activity and TNF-R with the compound and determining the effect on receptor release. Ribozymes and antisense molecules effective in altering expression of a TRRE promoter would decrease TNF-R release. Ribozymes and antisense molecules effective in altering expression of a TRRE inhibitor would increase TNF-R release.

Another screening method described in this disclosure is for testing the ability of polypeptides to modulate TRRE activity (Example 7). Cells expressing both TNF-R and a moderate level of TRRE activity are contacted with the test polypeptides, and the rate of receptor release is compared with the rate of spontaneous release. An increased rate of release indicates that the polypeptide is a TRRE promoter, while a decreased rate indicates that the polypeptide is a TRRE inhibitor. This assay can be used to test the activity of new polypeptides, and develop variants of polypeptides already known to modulate TRRE. The minimum size of polypeptide sequence that still encodes TRRE modulation activity can be determined by making a smaller fragment of the polypeptide and then using the screening assay to determine whether the activity is still present. Mutated and extended sequences can be tested in the same way.

Another screening method embodied in this invention is a method for screening substances that interfere with the action of a TRRE modulator at the protein level. The method involves incubating cells expressing TNF receptor

Either way, the rate of receptor release is compared in the presence and absence of the test substance, to identify compounds that enhance or diminish TRRE activity. Parallel experiments should be conducted in which the activity of the substance on receptor shedding is tested in the absence of added polypeptide (using cells that don't express the polypeptide). This will determine whether the activity of the test substance occurs via an effect on the TRRE promoter being added, or through some other mechanism.

This type of screening assay is useful for identifying antibodies that affect the activity of a TRRE modulator. Antibodies are raised against a TRRE modulator as described in the previous section. If the antibody decreases TRRE activity in the screening assay, then it has therapeutic potential to lower TRRE activity in vivo. Screening of monoclonal antibodies using this assay can also help identify binding or catalytic sites in the polypeptide.

This type of screening assay is also useful for high throughput screening of small molecule compounds that have the ability to affect the level of TNF receptors on a cell, by way of its influence on a TRRE modulator. Small molecule compounds that have the desired activity are often preferred for pharmaceutical compositions, because they are often more stable and less expensive to produce.

### *Medicaments and their use*

As described earlier, a utility of certain products embodied in this invention is to affect signal transduction from cytokines (particularly TNF). Products that promote TRRE activity have the effect of decreasing TNF receptors on the



surface of cells, which would decrease signal transduction from TNF. Conversely, products that inhibit TRRE activity prevent cleavage of TNF receptors, increasing signal transduction.

The ability to affect TNF signal transduction is of considerable interest in the management of clinical conditions in which TNF signaling contributes to the pathology of the condition. Such conditions include:

- Heart failure. IL-1 $\beta$  and TNF are believed to be central mediators for perpetuating the inflammatory process, recruiting and activating inflammatory cells. The inflammation depress cardiac function in congestive heart failure, transplant rejection, myocarditis, sepsis, and burn shock.
- Cachexia. The general weight loss and wasting occurring in the course of chronic diseases, such as cancer. TNF is believed to affect appetite, energy expenditure, and metabolic rate.
- Crohn's disease. The inflammatory process mediated by TNF leads to thickening of the intestinal wall, ensuing from lymphedema and lymphocytic infiltration.
- Endotoxic shock. The shock induced by release of endotoxins from gram-negative bacteria, such as E. coli, involves TNF-mediated inflammation
- Arthritis. TNF promotes expression of nitric oxide synthetase, believed to be involved in disease pathogenesis.

Other conditions of interest are multiple sclerosis, sepsis, inflammation brought on by microbe infection, and diseases that have an autoimmune etiology, such as Type I Diabetes.

Polypeptides of this invention that promote TRRE activity can be administered with the objective of decreasing or normalizing TNF signal transduction. For example, in congestive heart failure or Crohn's disease, the polypeptide is given at regular intervals to lessen the inflammatory sequelae.

The treatment is optionally in combination with other agents that affect TNF

signal transduction (such as antibodies to TNF or receptor antagonists) or that lessen the extent of inflammation in other ways.

Polynucleotides of this invention can also be used to promote TRRE activity by gene therapy. The encoding sequence is operably linked to control elements for transcription and translation in human cells. It is then provided in a form that will promote entry and expression of the encoding sequence in cells at the disease site. Forms suitable for local injection include naked DNA, polynucleotides packaged with cationic lipids, and polynucleotides in the form of viral vectors (such as adenovirus and AAV constructs). Methods of gene therapy known to the practitioner skilled in the art will include those outlined in U.S. Patent Nos. 5,399,346, 5,827,703, and 5,866,696.

The ability to affect TNF signal transduction is also of interest where TNF is thought to play a beneficial role in resolving the disease. In particular, TNF plays a beneficial role in the necrotizing of solid tumors. Accordingly, products of this invention can be administered to cancer patients to inhibit TRRE activity, thereby increasing TNF signal transduction and improve the beneficial effect.

Embodiments of the invention that inhibit TRRE activity include antisense polynucleotides. A method of conferring long-standing inhibitory activity is to administer antisense gene therapy. A genetic construct is designed that will express RNA inside the cell which in turn will decrease the transcription of the target gene (U.S. Patent No. 5,759,829). In humans, a more frequent form of antisense therapy is to administer the effector antisense molecule directly, in the form of a short stable polynucleotide fragment that is complementary to a segment of the target mRNA (U.S. Patent Nos. 5,135,917 and 5,789,573) — in this case, the transcript that encodes the TRRE modulator. Another embodiment of the invention that inhibits TRRE are ribozymes, constructed as described in an earlier section. The function of ribozymes in inhibiting mRNA translation is described in U.S. Patent Nos. 4,987,071 and 5,591,610.

Once a product of this invention is found to have suitable TRRE modulation activity in the in vitro assays described in this disclosure, it is preferable to also test its effectiveness in an animal model of a TNF mediated

disease process. Example 3 describes an LPS model for sepsis that can be used to test promoters of TRRE activity. Example 4 describes a tumor necrosis model, in which TRRE inhibitors could be tested for an ability to enhance necrotizing activity. Those skilled in the art will know of other animal models suitable for testing effects on TNF signal transduction or inflammation. Other illustrations are the cardiac ischemia reperfusion models of Weyrich et al. (*J. Clin. Invest.* 91:2620, 1993) and Garcia-Criado et al. (*J. Am. Coll. Surg.* 181:327, 1995); the pulmonary ischemia reperfusion model of Steinberg et al. (*J. Heart Lung Transplant.* 13:306, 1994), the lung inflammation model of International Patent Application WO 9635418; the bacterial peritonitis model of Sharar et al. (*J. Immunol.* 151:4982, 1993), the colitis model of Meenan et al. (*Scand. J. Gastroenterol.* 31:786, 1996), and the diabetes model of von Herrath et al. (*J. Clin. Invest.* 98:1324, 1996). Models for septic shock are described in Mack et al. *J. Surg. Res.* 69:399, 1997; and Seljelid et al. *Scand. J. Immunol.* 45:683-7.

For use as an active ingredient in a pharmaceutical preparation, a polypeptide, polynucleotide, or antibody of this invention is generally purified away from other reactive or potentially immunogenic components present in the mixture in which they are prepared. Typically, each active ingredient is provided in at least about 90% homogeneity, and more preferably 95% or 99% homogeneity, as determined by functional assay, chromatography, or SDS polyacrylamide gel electrophoresis. The active ingredient is then compounded into a medicament in accordance with generally accepted procedures for the preparation of pharmaceutical preparations, such as described in *Remington's Pharmaceutical Sciences 18th Edition* (1990), E.W. Martin ed., Mack Publishing Co., PA. Steps in the compounding of the medicament depend in part on the intended use and mode of administration, and may include sterilizing, mixing with appropriate non-toxic and non-interfering excipients and carriers, dividing into dose units, and enclosing in a delivery device. The medicament will typically be packaged with information about its intended use.

Mode of administration will depend on the nature of the condition being treated. For conditions that are expected to require moderate dosing and that are at well perfused sites (such as cardiac failure), systemic administration is acceptable. For example, the medicament may be formulated for intravenous administration, intramuscular injection, or absorption sublingually or intranasally. Where it is possible to administer the active ingredient locally, this is usually preferred. Local administration will both enhance the concentration of the active ingredient at the disease site, and minimize effects on TNF receptors on other tissues not involved in the disease process. Conditions that lend themselves to administration directly at the disease site include cancer and rheumatoid arthritis. Solid tumors can be injected directly when close to the skin, or when they can be reached by an endoscopic procedure. Active ingredients can also be administered to a tumor site during surgical resection, being implanted in a gelatinous matrix or in a suitable membrane such as Gliadel® (Guilford Sciences). Where direct administration is not possible, the administration may be given through an arteriole leading to the disease site. Alternatively, the pharmaceutical composition may be formulated to enhance accumulation of the active ingredient at the disease site. For example, the active ingredient can be encapsulated in a liposome or other matrix structure that displays an antibody or ligand capable of binding a cell surface protein on the target cell. Suitable targeting agents include antibodies against cancer antigens, ligands for tissue-specific receptors (e.g., serotonin for pulmonary targeting). For compositions that decrease TNF signal transduction, an appropriate targeting molecule may be the TNF ligand, since the target tissue may likely display an unusually high density of the TNF receptor.

Effective amounts of the compositions of the present invention are those that alter TRRE activity by at least about 10%, typically by at least about 25%, more preferably by about 50% or 75%. Where near complete ablation of TRRE activity is desirable, preferred compositions decrease TRRE activity by at least 90%. Where increase of TRRE activity is desirable, preferred compositions increase TRRE activity by at least 2-fold. A minimum effective amount of the

active compound will depend on the disease being treated, which of the TRRE modulators is selected for use, and whether the administration will be systemic or local. For systemic administration, an effective amount of activity will generally be an amount of the TRRE modulator that can cause a change in the enzyme activity by 100 to 50,000 Units — typically about 10,000 Units. The mass amount of protein, nucleic acid, or antibody is chosen accordingly, based on the specific activity of the active compound in Units per gram.

The following examples provided as a further guide to the practitioner, and are not intended to limit the invention in any way.

### EXAMPLES

#### Example 1: Assay system for TRRE activity.

This Example illustrates an assay system that measures TRRE activity on the human TNF-R in its native conformation in the cell surface membrane

Membrane-associated TNF-R was chosen as the substrate, as having microenvironment similar to that of the substrate for TRRE in vivo. Membrane-associated TNF-R also requires more specific activity, which would differentiate less-specific proteases. Cells expressing an elevated level of the p75 form of TNF-R were constructed by cDNA transfection into monkey COS-1 cells which express little TNF-R of either the 75 kDa or 55 kDa size.

The procedure for constructing these cells was as follows: cDNA of human p75 TNF-R was cloned from a  $\lambda$ gt10 cDNA library derived from human monocytic U-937 cells (Clontech Laboratories, Palo Alto, CA). The first 300 bp on both 5' and 3' ends of the cloned fragment was sequenced and compared to the reported cDNA sequence of human p75 TNF-R. The cloned sequence was a 2.3 kb fragment covering positions 58-2380 of the reported p75 TNF-R sequence, which encompasses the full length of the p75 TNF-R-coding sequence from positions 90-1475. The 2.3 kb p75 TNF-R cDNA was then subcloned into the multiple cloning site of the pCDNA3 eukaryotic expression vector. The

orientation of the p75 TNF-R cDNA was verified by restriction endonuclease mapping.

**Figure 1** illustrates the final 7.7 kb construct, pCDTR2. It carries the neomycin-resistance gene for the selection of transfected cells in G418, and the expression of the p75 TNF-R is driven by the cytomegalovirus promoter. The pCDTR2 was then transfected into monkey kidney COS-1 cells (ATCC CRL-1650) using the calcium phosphate-DNA precipitation method. The selected clone in G418 medium was identified and subcultured. This clone was given the designation **C75R**.

To determine the level of p75 TNF-R expression on C75R cells,  $2 \times 10^5$  cells/well were plated into a 24-well culture plate and incubated for 12 to 16 hours in 5% CO<sub>2</sub> at 37°C. They were then incubated with 2-30 ng <sup>125</sup>I human recombinant TNF (radiolabeled using the chloramine T method) in the presence or absence of 100-fold excess of unlabeled human TNF at 4°C for 2 h. After three washes with ice-cold PBS, cells were lysed with 0.1N NaOH and bound radioactivity was determined in a Pharmacia Clinigamma counter (Uppsala, Sweden).

**Figure 2** shows the results obtained. C75R had a very high level of specific binding of radiolabeled <sup>125</sup>I-TNF, while parental COS-1 cells did not. The number of TNF-R expressed on C75R was determined to be 60,000-70,000 receptors per cell by Scatchard analysis (Figure 2, inset). The K<sub>d</sub> value calculated was  $5.6 \times 10^{-10}$  M. This K<sub>d</sub> value was in close agreement to the values previously reported for native p75 TNF-R.

TRRE was obtained by PHA stimulation of THP-1 cells (WO 9802140). THP-1 cells (ATCC 45503) growing in logarithmic phase were collected and resuspended to  $1 \times 10^6$  cells/ml of RPMI-1640 supplemented with 1% FCS and incubated with  $10^{-6}$  M PMA for 30 min in 5% CO<sub>2</sub> at 37 °C. The cells were collected and washed once with serum-free medium to remove PMA and resuspended in the same volume of RPMI-1640 with 1% FCS. After 2 hours incubation in 5% CO<sub>2</sub> at 37°C, the cell suspension was collected, centrifuged, and the cell-free supernatant was collected as the source of TRRE.

In order to measure the effect of TRRE on membrane-bound TNF-R in the COS-1 cell constructs, the following experiment was performed. C75R cells were seeded at a density of  $2 \times 10^5$  cells/well in a 24-well cell culture plate and incubated for 12 to 16 hours at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$ . The medium in the wells was aspirated, replaced with fresh medium alone or with TRRE medium, and incubated for 30 min at  $37^\circ\text{C}$ . The medium was then replaced with fresh medium containing 30 ng/ml  $^{125}\text{I}$ -labeled TNF. After 2 hours at  $4^\circ\text{C}$ , the cells were lysed with 0.1 N NaOH and the level of bound radioactivity was measured. The level of specific binding of C75R by  $^{125}\text{I}$ -TNF was significantly decreased after incubation with TRRE. The radioactive count was 1,393 cpm on the cells incubated with TRRE compared to 10,567 cpm on the cells not treated with TRRE, a loss of 87% of binding capacity.

In order to determine the size of the p75 TNF-R cleared from C75R by TRRE, the following experiment was performed.  $15 \times 10^6$  C75R cells were seeded in a 150 mm cell culture plate and incubated at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  for 12 to 16 hours. TRRE medium was incubated with C75R cells in the 150 mm plate for 30 min and the resulting supernatant was collected and centrifuged. The concentrated sample was applied to 10% acrylamide SDS-PAGE and electrophoretically transferred to a polyvinylidene difluoride membrane (Immobilon). Immunostaining resulted in a single band of 40 kDa, similar to the size found in biological fluids. Thus, transfected COS-1 cells expressed high levels of human p75 TNF-R in a form similar to native TNF-R.

The following assay method was adopted for routine measurement of TRRE activity. C75R cells and COS-1 cells were seeded into 24-well culture plates at a density of  $2.5 \times 10^5$  cells/ml/well and incubated overnight (for 12 to 16 hours) in 5%  $\text{CO}_2$  at  $37^\circ\text{C}$ . After aspirating the medium in the well, 300  $\mu\text{l}$  of TRRE medium was incubated in each well of both the C75R and COS-1 plates for 30 min in 5%  $\text{CO}_2$  at  $37^\circ\text{C}$  (corresponding to A and C mentioned below, respectively). Simultaneously, C75R cells in 24-well plates were also incubated with 300  $\mu\text{l}$  of fresh medium or buffer. The supernatants were collected,

centrifuged, and then assayed for the concentration of soluble p75 TNF-R by ELISA.

ELISA assay for released TNF-R (WO 9802140) was performed as follows: Polyclonal antibodies to human p75 TNF-R were generated by immunization of New Zealand white female rabbits (Yamamoto et al. *Cell. Immunol.* 38:403-416, 1978). The IgG fraction of the immunized rabbit serum was purified using a protein G (Pharmacia Fine Chemicals, Uppsala, Sweden) affinity column (Ey et al. (1978) *Immunochemistry* 15:429-436, 1978). The IgG fraction was then labeled with horseradish peroxidase (Sigma Chemical Co., St. Louis, MO) (Tijssen and Kurstok, *Anal. Biochem.* 136:451-457, 1984). In the first step of the assay, 5 µg of unlabeled IgG in 100 µl of 0.05 M carbonate buffer (pH 9.6) was bound to a 96-well ELISA microplate (Corning, Corning, NY) by overnight incubation at 4°C. Individual wells were washed three times with 300 µl of 0.2% Tween-20 in phosphate buffered saline (PBS). The 100 µl of samples and recombinant receptor standards were added to each well and incubated at 37°C for 1 to 2 hours. The wells were then washed in the same manner, 100 µl of horseradish peroxidase-labeled IgG added and incubated for 1 hour at 37 °C. The wells were washed once more and the color was developed for 20 minutes (min) at room temperature with the substrates ABTS (Pierce, Rockford, IL) and 30% H<sub>2</sub>O<sub>2</sub> (Fisher Scientific, Fair Lawn, NJ). Color development was measured at 405 nm.

When C75R cells were incubated with TRRE medium, soluble p75 TNF-R was released into the supernatant which was measurable by ELISA. The amount of receptors released corresponded to the amount of TRRE added. There was also a level of spontaneous TNF-R release in C75R cells incubated with just medium alone. It is hypothesized that this is due to an endogenous source of proteolytic enzyme, a homolog of the human TRRE of monkey origin.

The following calculations were performed. A = (amount of soluble p75 TNF-R in a C75R plate treated with the TRRE containing sample); i.e. the total amount of sTNF-R in a C75R plate. B = (amount of soluble p75 TNF-R spontaneously released in a C75R plate treated with only medium or buffer



containing the same reagent as the corresponding samples but without exogenous TRRE); i.e. the spontaneous release of sTNF-R from C75R cells. C = (amount of soluble p75 TNF-R in a COS-1 plate treated with the TRRE sample or the background level of soluble p75 TNF-R released by THP-1.); i.e. the degraded value of transferred (pre-existing) sTNF-R in the TRRE sample during 30 min incubation in a COS-1 plate. This corresponds to the background level of sTNF-R degraded in a C75R plate. The net release of soluble p75 TNF-R produced only by TRRE activity existing in the initial sample is calculated as follows: (Net release of soluble p75 TNF-R only by TRRE) = A - B - C.

**Unit activity** of TRRE was defined as follows: 1 pg of soluble p75 TNF-R net release (A-B-C) in the course of the assay is one unit (U) of TRRE activity.

Using this assay, the time course of receptor shedding by TRRE was measured in the following experiment. TRRE-medium was incubated with C75R and COS-1 cells for varying lengths of time. The supernatants were then collected and assayed for the level of soluble p75 TNF-R by ELISA and the net TRRE activity was calculated. Detectable levels of soluble receptor were released by TRRE within 5 min and increased up to 30 min. Longer incubation times showed that the level of TRRE remained relatively constant after 30 min, presumably from the depletion of substrates. Therefore, 30 min was determined to be the optimal incubation time.

The induction patterns of TRRE and known MMPs by PMA stimulation are quite different. In order to induce MMPs, monocytic U-937 cells, fibrosarcoma HT-1080 cells, or peritoneal exudate macrophages (PEM) usually have to be stimulated for one to three days with LPS or PMA. On the other hand, as compared with this prolonged induction, TRRE is released very quickly in culture supernatant following 30 min of PMA-stimulation. The hypothesis that TRRE and sTNF-R form a complex *in vitro* was confirmed by the experiment that 25% TRRE activity was recovered from soluble p75 TNF-R affinity column. This means that free TRRE has the ability to bind to its catalytic product, sTNF-R. The remaining 75% which did not combine to the affinity column may already be

bound to sTNF-R or may not have enough affinity to bind to sTNF-R even though it is in a free form.

Example 2: Characterization of TRRE obtained from THP-1 cells .

5 TRRE obtained by PHA stimulation of THP-1 cells was partially purified from the culture medium (WO 9802140). First, protein from the medium was concentrated by 100% saturated ammonium sulfate precipitation at 4°C. The precipitate was pelleted by centrifugation at 10,000 x g for 30 min and resuspended in PBS in approximately twice the volume of the pellet. This  
10 solution was then dialyzed at 4°C against 10 mM Tris-HCl, 60 mM NaCl, pH 7.0. This sample was loaded on an anion-exchange chromatography, Diethylaminoethyl (DEAE)-Sephadex A-25 column (Pharmacia Biotech) (2.5 x 10 cm) previously equilibrated with 50 mM Tris-HCl, 60 mM NaCl, pH 8.0. TRRE was then eluted with an ionic strength linear gradient of 60 to 250 mM  
15 NaCl, 50 mM Tris-HCl, pH 8.0. Each fraction was measured for absorbance at 280 nm and assayed for TRRE activity. The DEAE fraction with the highest specific activity (the highest value of TRRE units/A280) was pooled and used in the characterizations of TRRE described in this example.

In the next experiment, the substrate specificity of the enzyme was  
20 elucidated using immunohistochemical techniques. Fluorescein isothiocyanate (FITC)-conjugated anti-CD54, FITC-conjugated goat anti-rabbit and mouse antibodies, mouse monoclonal anti-CD30, anti-CD11b and anti-IL-1R (Serotec, Washington D.C.) were used. Rabbit polyclonal anti-p55 and p75 TNF-R were obtained according to Yamamoto et al. (1978) *Cell Immunol.* 38:403–416. THP-  
25 1 cells were treated for 30 min with 1,000 and/or 5,000 U/ml of TRRE eluted from the DEAE-Sephadex column, and then transferred to 12 x 75 mm polystyrene tubes (Fischer Scientific, Pittsburgh, PA) at  $1 \times 10^5$  cells/100 $\mu$ l/tube. The cells were then pelleted by centrifugation at 350 x g for 5 min at 4°C and stained directly with 10 $\mu$ l FITC-conjugated anti-CD54 (diluted in cold PBS/0.5% sodium  
30 aside), indirectly with FITC-conjugated anti-mouse antibody after treatment of

mouse monoclonal anti-CD11b, IL-1R and CD30 and also indirectly with FITC-conjugated anti-rabbit antibody after treatment of rabbit polyclonal anti-p55 and p75 TNF-R.

THP-1 cells stained with each of the antibodies without treatment of TRRE were used as negative controls. The tubes were incubated for 45 min at 4°C, agitated every 15 min, washed twice with PBS/2% FCS, repelleted and then resuspended in 200µl of 1% paraformaldehyde. These labeled THP-1 cells were analyzed using a fluorescence activated cell sorter (FACS) (Becton-Dickinson, San Jose, CA) with a 15 mW argon laser with an excitation of 488 nm. Fluorescent signals were gated on the basis of forward and right angle light scattering to eliminate dead cells and aggregates from analysis. Gated signals (10<sup>4</sup>) were detected at 585 BP filter and analyzed using Lysis II software. Values were expressed as percentage of positive cells, which was calculated by dividing mean channel fluorescence intensity (MFI) of stained THP-1 cells treated with TRRE by the MFI of the cells without TRRE treatment (negative control cells).

To test the *in vitro* TNF cytolytic assay by TRRE treatment the L929 cytolytic assay was performed according to the method described by Gatanaga et al. (1990b). Briefly, L929 cells, an adherent murine fibroblast cell line, were plated (70,000 cells/0.1ml/well in a 96-well plate) overnight. Monolayered L929 cells were pretreated for 30 min with 100, 500 or 2,500 U/ml of partially-purified TRRE and then exposed to serial dilutions of recombinant human TNF for 1 hour. After washing the plate with RPMI-1640 with 10% FCS to remove the TRRE and TNF, the cells were incubated for 18 hours in RPMI-1640 with 10% FCS containing 1 µg/ml actinomycin D at 37°C in 5% CO<sub>2</sub>. Culture supernatants were then aspirated and 50 µl of 1% crystal violet solution was added to each well. The plates were incubated for 15 min at room temperature. After the plates were washed with tap water and air-dried, the cells stained with crystal violet were lysed by 100 µl per well of 100 mM HCl in methanol. The absorbance at

550 nm was measured using an EAR 400 AT plate reader (SLT-Labinstruments, Salzburg, Austria).

To investigate whether TRRE also truncates the ~55 kDa size of TNF-R, partially-purified TRRE was applied to THP-1 cells which express low levels of both p55 and p75 TNF-R (approximately 1,500 receptors/cell by Scatchard analysis). TRRE eluate from the DEAE-Sephadex column was added to THP-1 cells ( $5 \times 10^6$  cells/ml) at a final TRRE concentration of 1,000 U/ml for 30 min. The concentration of soluble p55 and p75 TNF-R in that supernatant was measured by soluble p55 and p75 TNF-R ELISA. TRRE was found to truncate both human p55 and p75 TNF-R on THP-1 cells and released 2,382 and 1,662 pg/ml soluble p55 and p75 TNF-R, respectively.

Therefore, TRRE obtained by PHA stimulation of THP-1 cells is capable of enzymatically cleaving and releasing human p75 TNF-R on C75R cells, and both human p55 and p75 TNF-R on THP-1 cells.

Partial inhibition of TRRE activity was obtained by chelating agents such as 1,10-phenanthroline, EDTA and EGTA (% TRRE activity remaining were 41%, 67% and 73%, respectively, at 2 mM concentration). On the other hand, serine protease inhibitors such as PMSF, AEBSF and 3,4-DCI, and serine and cysteine protease inhibitors such as TLCK and TPCK had no effect on the inhibition of TRRE. TRRE was slightly activated in the presence of  $Mn^{2+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ , and  $Co^{2+}$  (% TRRE activities remaining were 157%, 151%, 127%, and 123%, respectively), whereas partial inhibition occurred in the presence of  $Zn^{2+}$  and  $Cu^{2+}$  (% TRRE activities remaining were 23% and 47%, respectively) (WO 9802140).

TRRE fractions from the most active DEAE fraction (60 mM to 250 mM NaCl) can be purified further. In one method (WO 9802140), the fractions were concentrated to 500  $\mu$ L with a Centriprep-10 filter (10,000 MW cut-off membrane) (Amicon). This concentrated sample was applied to 6% PAGE under non-denaturing native conditions. The gel was sliced horizontally into 5 mm strips and each was eluted into 1 ml PBS. The eluates were then tested according to the assay (Example 1) for TRRE activity.

### Example 3: TRRE activity alleviates septic shock

The following protocol was used to test the effects of TRRE in preventing mortality in a model for septic shock. Mice were injected with lethal or sublethal levels of LPS, and then with a control buffer or TRRE. Samples of peripheral blood were then collected at intervals to establish if TRRE blocked TNF-induced production of other cytokines in the bloodstream. Animals were assessed for the ability of TRRE to block the clinical effects of shock, and then euthanized and tissues examined by histopathological methods.

Details were as follows: adult Balb/c mice, were placed in a restraining device and injected intravenously via the tail vein with a 0.1 ml solution containing 10 ng to 10 mg of LPS in phosphate buffer saline (PBS). These levels of LPS induce mild to lethal levels of shock in this strain of mice. Shock results from changes in vascular permeability, fluid loss, and dehydration, and is often accompanied by symptoms including lethargy, a hunched, stationary position, rumped fur, cessation of eating, cyanosis, and, in serious cases, death within 12 to 24 hours. Control mice received an injection of PBS. Different amounts (2,000 or 4,000 U) of purified human TRRE were injected IV in a 0.1 ml volume within an hour prior to or after LPS injection. Serum (0.1 ml) was collected with a 27 gauge needle and 1 ml syringe IV from the tail vein at 30, 60 and 90 minutes after LPS injection. This serum was heparinized and stored frozen at  $-20^{\circ}\text{C}$ . Samples from multiple experiments were tested by ELISA for the presence of sTNF-R, TNF, IL-8 and IL-6. Animals were monitored over the next 12 hours for the clinical effects of shock. Selected animals were euthanized at periods from 3 to 12 hours after treatment, autopsied and various organs and tissues fixed in formalin, imbedded in paraffin, sectioned and stained by hematoxylin-eosin (H and E). Tissue sections were subjected to histopathologic and immunopathologic examination.

**Figure 3** shows the results obtained. (♦) LPS alone; (■) LPS plus control buffer; (●) LPS plus TRRE (2,000 U); (▲) LPS plus TRRE (4,000 U).

Mice injected with LPS alone or LPS and a control buffer died shortly after injection. 50% of the test animals were dead after 8 hours (LPS) or 9 hours (LPS plus control buffer), and 100% of the animals were dead at 15 hours. In contrast, animals treated with TRRE obtained as described in Example 1 did much better. When injections of LPS were accompanied by injections of a 2,000 U of TRRE, death was delayed and death rates were lower. Only 40% of the animals were dead at 24 hours. When 4,000 U of TRRE was injected along with LPS, all of the animals had survived at 24 hours. Thus, TRRE is able to counteract the mortality induced by LPS in test animals.

Example 4: TRRE activity decreases tumor necrotizing activity

The following protocol was followed to test the effects of TRRE on tumor necrosis in test animals in which tumors were produced, and in which TNF was subsequently injected.

On Day 0, cutaneous Meth A tumors were produced on the abdominal wall of fifteen BALB/c mice by intradermal injection of  $2 \times 10^5$  Meth A tumor cells. On Day 7, the mice were divided into three groups of five mice each and treated as follows:

- Group 1: Injected intravenously with TNF ( $1 \mu\text{g}/\text{mouse}$ ).
- Group 2: Injected intravenously with TNF ( $1 \mu\text{g}/\text{mouse}$ ) and injected intratumorally with TRRE obtained as in Example 1 (400 units/mouse, 6, 12 hours after TNF injection).
- Group 3: Injected intravenously with TNF ( $1 \mu\text{g}/\text{mouse}$ ) and injected intratumorally with control medium (6, 12 hours after TNF injection).

On Day 8, tumor necrosis was measured with the following results: Group 1: 100% of necrosis (5/5); Group 2: 20% (1/5); Group 3: 80% (4/5). Injections of TRRE greatly reduced the ability of TNF to induce necrosis in Meth A tumors in BALB/c mice.

Since adding TRRE activity ablates the beneficial necrotizing activity of TNF, blocking endogenous TRRE activity would promote the beneficial effects of TNF.

5 **Example 5: Nine new polynucleotide clones that affect TRRE activity**

A number of cells have been found to express high levels of TRRE activity, especially after PMA stimulation. These include the cell lines designated THP-1, U-937, HL-60, ME-180, MRC-5, Raji, K-562. Jurkat cells have a high TRRE activity (850 TRRE U/mL at  $10^{-2}$  PMA). In this experiment, the expression  
10 library of the Jurkat T cell (ATCC #TIB-152) was obtained and used to obtain 9 polynucleotide clones that augment TRRE activity.

Selection of expression sequences in the library was done by repeated cycles of transfection into COS-1 cells, followed by assaying of the supernatant as in Example 1 for the presence of activity cleaving and releasing the TNF  
15 receptor. Standard techniques were used in the genetic manipulation. Briefly, the DNA of  $10^6$  Jurkat cells was extracted using an InVitrogen plasmid extraction kit according to manufacturer's directions. cDNA was inserted in the ZAP Express™/EcoRI vector (cat. no. 938201, Stratagene, La Jolla CA. The library was divided into 48 groups of DNA and transformed into COS-1 cells using the  
20 CaCl transfection method. Once the cells were grown out, the TRRE assay was performed, and five positive groups were selected. DNA from each of these five groups was obtained, and transfected into *E. coli*, with 15 plates per group. DNA was prepared from these cells and then transfected into COS-1 cells once more. The cells were grown out, and TRRE activity was tested again. Two positive  
25 groups were selected and transfected into *E. coli*, yielding 98 colonies. DNA was prepared from 96 of these colonies and transfected into COS-1 cells. The TRRE activity was performed again, and nine clones were found to substantially increase TRRE activity in the assay. These clones were designated 2-8, 2-9, 2-14, 2-15, P2-2, P2-10, P2-13, P2-14, and P2-15.

30 **Figure 4** is a bar graph showing the TRRE activity observed when the 9 clones were tested with C75 cells in the standard assay (Example 1).

These nine clones were then sequenced according to the following procedure:

1. Plasmid DNA was prepared using a modified alkaline lysis procedure.
2. DNA sequencing was performed using DyeDeoxy termination reactions (ABI). Base-specific fluorescent dyes were used as labels.
3. Sequencing reactions were analyzed on 5.75% Long Ranger™ gels by an ABI 373A-S or on 5.0% Long Ranger™ gels by an ABI 377 automated sequencer.
4. Subsequent data analysis was performed using Sequencher™ 3.0 software.

Standard primers T7X, T3X, -40, -48 Reverse, and BK Reverse (BKR) were used in sequencing reactions. For each clone, several additional internal sequencing primers (listed below) were synthesized.

NCBI BLAST (Basic Local Alignment Search Tool) sequence analysis (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410) was performed to determine if other sequences were significantly similar to these sequences. Both the DNA sequences of the clones and the corresponding ORFs (if any) were compared to sequences available in databases.

The following clones were obtained and sequenced:



TABLE 1: DNA sequences affecting TRRE activity					
Clone	Sequence Designation	SEQ ID NO:	Approx Length (bp)	Expression Designation	Related sequences (potential homology)
2-9	AIM2	1	4,047		—
2-8	AIM3T3 (partial sequence)	2	739		<i>M. musculus</i> 45S pre-rRNA gene
	AIM3T7 (partial sequence)	3	233		
2-14	AIM4	4	2,998	Mey3	human arfaptin 2 and others (see below)
2-15	AIM5	5	4,152		—
P2-2	AIM6	6	3,117	Mey5	—
P2-10	AIM7	7	3,306	Mey6	Human Insulin-like Growth factor II Receptor
P1-13	AIM8	8	4,218		—
P2-14	AIM9	9	1,187	Mey8	—
P2-15	AIM10	10	3,306		E1b-55kDa-associated protein

Clone 2-9 (AIM2): The internal primers used for sequencing are shown in SEQ. ID NOS:11-38. The sequence of AIM2 is presented in SEQ ID NO:1. The complementary strand of the AIM2 sequence is SEQ ID NO:147. The longest open reading frame (ORF) in the AIM2 sequence is 474 AA long and represented in SEQ ID NO:148.

Clone 2-8 (AIM3): Two partial sequences of length 739 and 233 were obtained and designated AIM3T3 and AIM3T7. The internal primers used for sequencing are shown in SEQ. ID NOS:39-46. The sequences of AIM3T3 and

AIM3T7 are presented in SEQ ID NOs:2 and 3, respectively. The BLAST search revealed that the AIM3T3 sequence may be homologous to the mouse (*M. musculus*) 28S ribosomal RNA (Hassouna et al. *Nucleic Acids Res.* 12:3563-3583, 1984) and the *M. musculus* 45S pre-rRNA genes (Accession No. X82564.

- 5 The complementary sequence of the AIM3T3 sequence showed 99% similarity over 408 bp beginning with nt 221 of SEQ ID NO:2 to the former and 97% similarity over the same span to the latter.

*Clone 2-14 (AIM4).* The internal primers used for sequencing are shown in SEQ. ID NOS:14-65. The sequence of AIM4 is presented in SEQ ID NO:4. The  
10 complementary strand of the AIM4 sequence is SEQ ID NO:149. The longest ORF in the AIM4 sequence is 236 AA long and represented in SEQ ID NO:150. AIM4 has significant alignments to human sequences arfaptin 2, ADE2H1 mRNA showing homologies to SAICAR synthetase, polypyrimidine tract binding protein (heterogeneous nuclear ribonucleoprotein I) mRNA, several PTB genes for  
15 polypirimidine tract binding proteins, mRNA for por1 protein. Human arfaptin 2 is a putative target protein of ADP-ribosylation factor that interacts with RAC1 by binding directly to it. RAC1 is involved in membrane ruffling. Arfaptin 2 has possible transmembrane segments, potential CK2 phosphorylation sites, PKC phosphorylation site and RGD cell attachment sequence.

20 *Clone 2-15 (AIM5):* The internal primers used for sequencing are shown in SEQ. ID NOS:66-80. The sequence of AIM5 is presented in SEQ ID NO:5. The BLAST search revealed that the AIM5 sequence displays some similarity to Human Initiation Factor 5A (eIF-5A) Koettnitz et al. (1995) *Gene* 159:283-284, 1995 and Human Initiation Factor 4D (eIF 4D) Smit-McBride et al. (1989) *J. Biol.*  
25 *Chem.* 264:1578-1583, 1989.

*Clone P2-2 (AIM6):* The internal primers used for sequencing are shown in SEQ. ID NOS:81-93. The sequence of AIM6 is presented in SEQ ID NO:6. The longest ORF in the AIM6 sequence is 1038 AA long and represented in SEQ ID NO:151.

30 *Clone P2-10 (AIM7):* The internal primers used for sequencing are shown in SEQ. ID NOS:94-106. The sequence of AIM7 is presented as SEQ ID NO:7.

5 The longest ORF in the AIM7 sequence is 849 AA long and represented in SEQ ID NO:152. The BLAST search revealed that this clone may be related to the Human Insulin-like Growth Factor II Receptor (Morgan et al. *Nature* 329:301-307, 1987 or the Human Cation-Independent Mannose 6-Phosphate Receptor mRNA (Oshima et al. *J. Biol. Chem.* 263:2553-2562, 1988). The AIM7 sequence showed roughly 99% identity to both sequences over 2520 nucleotides beginning with nt 12 of SEQ ID NO:7 and 99% similarity to the latter over the same span.

10 *Clone P2-13 (AIM8)*: The internal primers used for sequencing are shown in SEQ. ID NOS:107-118. The sequence of AIM8 is presented as SEQ ID NO:8. The longest ORF in the AIM8 sequence is 852 AA long and represented in SEQ ID NO:153.

*Clone P2-14 (AIM9)*: The internal primers used for sequencing are shown in SEQ. ID NOS:119-124. The sequence of AIM9 is presented as SEQ ID NO:9. The longest ORF was about 149 amino acids in length.

15 *Clone P2-15 (AIM10)*: The internal primers used for sequencing are shown in SEQ. ID NOS:125-146. The sequence of AIM10 is presented as SEQ ID NO:10. The longest ORF in the AIM10 sequence is 693 AA long and represented in SEQ ID NO:154. Sequence 10 on BLASTN search of non-redundant databases at NCBI aligns with Human mRNA for E1b-55kDa-associated protein, locus HSA7509 (Accession AJ007509, NID g3319955).

20 Clonal DNA may be directly injected into test animals in order to test the ability of these nucleic acids to induce TRRE activity, counteract septic shock and/or affect tumor necrosis, as is described in detail in Examples 3 and 4. Alternatively, proteins or RNA can be generated from the clonal DNA for similar testing.

#### Example 6: Expression of newly obtained clones

30 Example 5 describes 9 new clones which enhance TRRE activity in a cell surface assay system. The clones were obtained in the pBK-CMB Phagmid vector .

The following work was done on contract through the commercial laboratory Lark Technologies, Houston, TX. The clones were removed from shuttle vectors and inserted into expression vectors in the following manner. Recombinant plasmid (pBK-CMV containing insert) was digested with appropriate restriction enzyme(s) such as Spe I, Xba I, EcoR I or others, as appropriate. The Baculovirus Transfer Vector (pAcGHLT-A Baculovirus Transfer Vector, PharMingen, San Diego, CA, Cat. No. 21460P) was also cut with appropriate restriction enzyme(s) within or near the multiple cloning site to receive the insert removed from the shuttle vector.

The fragment of interest being subcloned was isolated from the digest using Low-Melting agarose electrophoresis and purified from the gel using a Qiaquick Gel Extraction Kit following Lark SOP MB 020602. If necessary, the receiving vector was treated with alkaline phosphatase according to Lark SOP MB 090201. The fragment was ligated into the chosen site of the vector pAcGHLT-A. The recombinant plasmid was transformed into *E. coli* XL1 Blue MRF' cells and the transformed bacterial cells were selected on LB agar plates containing ampicillin (100µg/ml). Ampicillin resistant colonies were picked and grown on LB broth containing ampicillin for plasmid preparation.

Plasmid DNA was prepared using Alkaline Minilysate Procedure (Lark SOP MB 010802 and digested with appropriate restriction enzyme(s). Selected subclones were confirmed to be of the correct size. Subclones were digested with other appropriate restriction enzyme(s) to ascertain correct orientation of the insert by confirming presence of fragments of proper size(s). A subclone was grown in 100 ml of LB broth containing ampicillin (100µg/ml) and the plasmid DNA prepared using Qiagen Midi Plasmid Preparation Kit (Lark SOP MB 011001). The DNA concentration was determined by measuring the absorbance at 260 nm and the DNA sample was verified to be originated from correct subclone by restriction digestion.

Thus were produced the expression constructs for Mey3, Mey5, Mey6, Mey8 now with the coding sequence of interest fused to GST gene with polyhistidine tag, protein kinase A site and thrombin cleavage site. The GST

gene and now the fusion protein are under the polyhedrin promotor. PharMingen (San Diego, CA) incorporated the vector with insert into functional baculovirus particles by co-inserting the transfer vector (pAcGHLT) into susceptible insect cell line S along with linearized virus DNA (PharMingen, San Diego, CA, 5 BaculoGold viral DNA, Cat. No. 21100D). The functional virus particles were grown again on the insect cells to generate a high titer stock. Protein production was then done by infecting a large culture of cells in Tini cell. The cells were harvested when the protein yield reached a maximum and before the virus killed the cells. Fusion proteins were collected on a glutathione-agarose column, 10 washed and released with glutathionine.

Proteins collected from the affinity column were quantified by measuring OD<sub>280</sub> and were assayed on gels using SDS-PAGE and Western blotting with labeled anti-GST (PharMingen, San Diego, CA, mAbGST Cat. No. 21441A) to confirm that all the bands present included the GST portion.

15 Four of the ten sequences have been cloned, expressed in baculovirus infected insect cells, and then purified.

TABLE 2: Expressed protein from Jurkat library clones		
Name	Sequence in insert	Amount of protein (mg/mL)
Mey3	AIM4	4.7, 5.0
Mey5	AIM6	1.36, 1.50
Mey6	AIM7	0.33
Mey8	AIM9	1.53

Gels indicated the presence of the GST protein in addition to larger proteins that were also positive with the anti-GST antibody in Western analyses. 20 Mey3 repeatedly exhibited the presence of proteins around 32kDa, 56kDa, bands around 60-70kDa and another larger than 70kDa. Mey5 consistently had proteins migrating as approximately 34kDa, 38kDa, 58kDa, around 60-70kDa, and others larger than 70kDa. Mey6 had protein bands around 34kDa, 56kDa,

58kDa, and bands around 60-70kDa. Mey8 had protein bands around 36kDa, 58kDa and bands around 60-70kDa. All of the indicated bands were positive for GST. The bands may represent the desired fusion protein or degradation/cleavage product generated during growth and purification.

5

**Example 7: Assay of expression products for effect on TNF-R cleaving activity**

The following method was used to measure TRRE activity of Mey 3, 5, 6 and 8. C75R cells and COS-1 cells were seeded into 24-well culture plates at a density of  $2.5 \times 10^5$  cells/ml/well and incubated overnight (for 12 to 16 hours) in 5% CO<sub>2</sub> at 37°C. After aspirating the medium in the well, 300µl of 1 µg of Mey 3, 5 and 8 were incubated in each well of both the C75R and COS-1 plates for 30 min in 5% CO<sub>2</sub> at 37°C (corresponding to A and C mentioned below, respectively). Simultaneously, C75R cells in 24-well plates were also incubated with 300µl of fresh medium or buffer (corresponding to B mentioned below). The supernatants were collected, centrifuged, and then assayed for the concentration of soluble p75 TNF-R by ELISA as described in Example 1.

The following results were obtained:

<b>TABLE 3: Enzymatic activity of expressed clones</b>	
<b>Clone No.</b>	<b>TNF-receptor releasing activity U/mg</b>
Mey-3	341
Mey-5	671
Mey-6	452
Mey-8	191

20

Example 8: Effectiveness of expression products in treating septic shock

The protocol outlined in Example 3 was used to test the effects of the expression products from the new clones in preventing mortality in the septic shock model.

5 Different amounts of recombinant Mey 3, 5, and 8 (10 – 100 ug/mouse) were injected i.v. in a 0.05 ml volume within an hour prior to or after injection of a lethal dose of LPS. Serum (0.1ml) was collected using a 27 gauge needle and 1 ml syringe from the tail vein at 30, 60 and 90 minutes after LPS injection. This serum was heparinized and stored frozen at -20°C. Samples from multiple  
10 experiments were tested by ELISA for the presence of solubilized TNR-R, the TNR ligand, IL-8, and IL-6. Animals were monitored over the next 12 hours for the clinical effects of shock. Selected animals were euthanized from 3 to 12 hours after treatment, autopsied and various organs and tissues fixed in formalin, imbedded in paraffin, sectioned and stained by hematoxylin-eosin (H and E).  
15 Tissue sections were subjected to histopathologic and immunopathologic examination.

**Figure 5** shows the results obtained. (♦) saline; (■) BSA; (Δ) Mey-3 (100 µg); (X) Mey-3 (10 µg); (\*) Mey-5 (10 µg); (●) Mey-8 (10 µg).

Mice injected with LPS alone or LPS, a control buffer or control protein  
20 (BSA) died rapidly. All of the animals in this group were dead at 24 hours. In contrast, when injections of LPS were accompanied by injections of a 10 – 100 ug of Mey 3, 5 and 8, death was delayed and death rates were lower. None of the animal were dead at 24 hours that had been treated with Mey 3 and Mey 5. Only 66 % of the animals were dead at 24 hours that had been treated with Mey  
25 8. Thus, Mey 3, 5 and 8 were able to counteract the mortality induced by LPS in test animals.

## SEQUENCE LISTING

### (1) GENERAL INFORMATION:

- (i) APPLICANT: Gatanaga, T.  
Granger, G.A.
- (ii) TITLE OF INVENTION: Factors Altering Tumor Necrosis  
Factor Receptor Releasing Enzyme Activity
- (iii) NUMBER OF SEQUENCES: 154
- (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: MORRISON & FOERSTER  
(B) STREET: 755 PAGE MILL ROAD  
(C) CITY: Palo Alto  
(D) STATE: CA  
(E) COUNTRY: USA  
(F) ZIP: 94304-1018
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Diskette  
(B) COMPUTER: IBM Compatible  
(C) OPERATING SYSTEM: Windows  
(D) SOFTWARE: FastSEQ for Windows Version 2.0b
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: USSN 09/081,385  
(B) FILING DATE: 014-NOV-1998
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME:  
(B) REGISTRATION NUMBER:  
(C) REFERENCE/DOCKET NUMBER: 22000-20577.21
- (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 650-813-5600  
(B) TELEFAX: 650-494-0792  
(C) TELEX: 706141

### (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 4047 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGCTTTT	CTTCTCTC	CCGGGAAAG	CCGGGGCCAG	AGACCCGCAC	TCGGACCAGG	60
CGGGGGCTGC	GGGGCCAGAG	TGGGCTGGGG	AGGGCTGGGA	GGGCGTCTGG	GGCCGGCTCC	120
TCCAGGCTGG	GGGCCGCCAG	CTCCGGGAAG	GCAGTCCTGG	CCTGCGGATG	GGGCCGCGCG	180
TGGGGCCCGG	CGGGGCGGCC	TCGGGAGGCG	TCCAGGCTGC	GGGAGCGGGA	GGAGCGGCCG	240
TGCGGGCGCC	AGCGCCGTGG	GTGGAGGTCG	CCGTCCCTCC	TGAGGGGCAG	CCAGTGCCTT	300
TGGGACCCGG	GAGCAGAGCC	CGCGCTCC	CAGCGGCCTC	CCCGGGGGTC	TCACCGGGTC	360
ACCCGAGAGC	GGAGGCCCGC	GCTCCGCAGA	AACCCGGGGC	GGCGCGGGG	AAGCAGCGCC	420
CTCAGGCGTC	GGAGGAGCCC	CCAGAAGGAC	CTCGCGCTT	CCCGCCGGG	TCCGACCGCC	480
TGGGTTCCGT	GCGGGACGGC	CCAGGCCGCC	AGGACCCCA	AGCGCAGCTC	AGTCTGCGGG	540
GCACGACCCA	GAGGCCAGCA	GCAGAGGACG	GGGCCGGGGC	CGGGAGAGGG	CGGGGAGGGC	600
GCTCCTGGGA	GGTCAAGGCC	AGGGCTAGAC	TTTCAGGGTC	ATGGCCTGGC	CCCTCATCCC	660
CAGGGAGGTG	AGGGGGCTCT	GTGAGCAGAG	GGGGCCCCGG	TGGAGAAGGC	GCTGCTAGCC	720



```

AGGGGCGGGG CAGGAGCCCA GGTGGGGACT TAAGGGTGGC TGAAGGGACC CTCAGGCTGC 780
AGGGATAGGG AGGGAAGCTA GGGGTGTGGC TTGGGGAGGT GCTGGGGGAC CGCGGGCGCC 840
CTTTATTCTG AAGCCGAATG TGCTGCCGGA GTCCCCAGTG ACCTAGAAAT CCATTTCAAG 900
ATTTTCAGGA GTTTCAGGTG GAGACAAAGG CCAGGCCAGG GTGAAAATGT GGCAGTGACA 960
GAGTATGGGG TGAGAACCAC GGAGAGAGGA AGTCCCGAG GCGGATGATG GGACAGAGAG 1020
CGGGGACCAG AATTTTTTAA AACGCATCTG AGATGCGTTT GGCAGACTCA TAGTTGTTTT 1080
CCTTTACGCG AGAAAGTGTG GGCAGAAAGC AGCTCTAAAG CCCAGGCTGC CCAGCCTGCA 1140
CTGGCAGAGC TGACGGAAGG CCAGGGCAGA GCCTTCCCTC CCTGTACAG ACATGAGCCC 1200
TGGAGATCTG GAATGAGGCA GATGTGCCCA GGGAAAGCTG ATCCGCCCGG ACCCAGGGCC 1260
CCCCGGGTGC CCCTTTGAGC GTGGAATCGT TGCCAGGTCA TGGCTCCCTG CTATCGAACA 1320
CCGGACACGG GTCGTGTGCT GCACCTGGCA GTTGACGAGC CGACACCCAC AATGCCTTAA 1380
GAGGTGATGA CTGCCTTCCA GGGGCTGGC TGGCTGACAC TTTGCATGGC TCCTGGAGAA 1440
GAGGGATTGA GTGGAGTCCA CGGGTCATGG CCACGTCTTG GGTGCTGCCT CTGAGGCAGG 1500
GCCCGGCTGG GGTGAGAAAG GGTGAGAGAC AGGTTCCTGC CAGTTACGCC TCTAACCGGT 1560
GGTCTTACG CCTAGGAACC CACTGGGGGC TTATGAAACT GCAGGTGGCT GAGTCTTTGC 1620
CATGGGGTCT CTCCTTCAGG AGGTCTGGGT GGGGCCGGAG ACTGTACCCC ACAAGGGGTC 1680
CCAGGTGAGG CGGATGTGGC CTGGCGCTGT GTGGCTCTGG ACCTAGTCCT TGGGCTTGGG 1740
CTGGCGCCCA GGGCCTGGGC TTGAGACAGC TGTGACGAGC GCAAGCCATT TACCCGTTT 1800
GTGGGGACAT TACATCTTCC TAGCTTGAA CACACAGGCA GCCAGGGTTG TTATCCACAT 1860
TCCTCTCCA TGTCTTCTC TTGAGAACTT TTACCAGGTA TGTACGAGC TGGGCTCCAC 1920
CAGGGAGACT CAAGTGGAAA GCCTCATCC TTGTCTCCA GGAGACAGGA AAACCTATGG 1980
TTACAATTCC AGGACAAGA GCGATGCATG TGAGGTGTGG CAAATCTCAC TGTCAACTG 2040
GAGAAATCAG AGACAGCTTC CTGGAGGCAG TGACACCTGG ACAGGCTTCT CCACAGGAGG 2100
AAGCGAGTGA GAGAAGCCAA CTGGGATGGA CCCATCATGT AGGGGGAACA GTGCGCGCAG 2160
AACCAACAAC CACCCCAACC CTAGGCCAG AGCTCACGGA GAGAGCTGGG CCTCTCGGGG 2220
TGACTACATA GTTCCCTGCT GGTCTTAGG TCTTGTCTT GGGCAGCTCT GCTGAGACCT 2280
CTATGCTGT TCCAGGCTGC ACCAAGGTTT TGTGACTATT GGTCTGGGGT TGTTTTGCAG 2340
CAACTGAAGT GTTCTGTGT AAAACAGGCA CTGTATTTC TGGAAGGAAT GCTGTTTGT 2400
CTTGCTGCCA CAAACATTGA GCAGCATTTA GTGGGCGGTT TATATCTTGT GGAGTAATGG 2460
GTGTTTTTGA AGTCTGTCT GGGTACTGCA CATTAAAAGG AATATCATT TCTGAAACAT 2520
TGCTATTTTC CACACCAGAA ATCATATCCT CTGTCTGTC CATGTCTGAA GACCTTACAC 2580
GAGAAAGTCT TAATGTAAGT TTAGTAGAGT CCTTGGATGG AGAACTAAT ATATCATACA 2640
TTGCCGCTTT CTCACTCTGC TCTTTTTCAT CCTTGCTAA TTTCAATTT TCTGCTTCT 2700
TTTGTTTTCT TTCTGGAGAA TCTAGCAAGA TATCTGGTGG AACATCTCGA GGTGATGAAC 2760
AAGGTAGAGA CTGAGATTGT AGGATTAAG GTGGTCTTGA GCCTTTAGGA GTTCCTTCAC 2820
TTCCAGCAGG GGAGCATACT GGCTGTGGAG ATCTCAAGGG AAAAGATGCA GCATTCCTCA 2880
TTGTGAAGA ATCTCCATCG TCACTACTTA GCCTGTGCAC CATGTGTAGG TAGTCTCAC 2940
TTGAACCATG TCTAGGATTA TCAGCATGAT GATTAGCTGA ATTGCCAGAC AACGGACCAG 3000
AAACTTTATT ATCATGTATG TTTCTCAAAC CACCTGCAAC AATGGGACTT GATACCGATG 3060
CTTGTGTCAT CTGTGGATGT GTTGTGTAAC TTGAAGGATG GGAATATGGC ATGTATCCTG 3120
CAGGGCTTTG TGGGGCGTAT GGACTAGGCA CTGGGCTATT TTGCTGTGGC ATAAATCTGT 3180
TCCCAGAGCT TGTCTGTGGT GGCACAAACC GGCTGGAGGG GCTATGTGAG ATAGTGGTTT 3240
GTTGATAATT GGAAGATGCA GGACTACTGT GCATGGAATT CTGAGAAAGT TTATACTGAG 3300
ACATCATCAT TCCACTTTGT ACATATCTGT TCTGCATGCT TTTCTCCCTG AAAACATTAG 3360
GACTCCTTGC CAGGACGGCC TGCAACAAGA CTGGTATGTC ACCTTCTGGG TCATCACTGC 3420
CAAGGTTATC TTTCAACTCT ATGTGATCTG TTGATACCTG GTTGAGGCTA TGGACAAGCT 3480
GTGAAACCAA ATTGTATCC CTACAAGCCA AAAGGCAGTT CACCTCTTCT GCTATTCGTG 3540
CATTAAGAG AAGGCTCTTT GTAGTTGTAG CAGGTAAAGG AGATGGAAGA GGCAGCTGGT 3600
TCAGGAGGTC TGTGAGACTA GCAATCCCCG CAAGAGTAGT AATGGGGACA TGGGGCATAT 3660
CCCCATTCT CATGAAATTC TGGAAATGGT TTGCCTATAA AAGTACTTAG TTCAGGTGCC 3720
AGCTGTCTAT ACTTCCCAT TCCCAAACAC TGGGCGAATC GGCCTCTGAA TCCAAGGGGA 3780
GGCCGAGGCC GCTGTGGCGA GAGACTATAA TCCGGGCCGG GAGGGGGGGG GGCTACGGCT 3840
CCTCTTCCGT CTCCTCAGTG CGGGGAACAT GTAGAGCCGG GGGGAGACCA GCCGAGAAGA 3900
CAAATCGTTG CTCTTCTTC CTCCTCTCC TCCTTCTCCC ACATAGAAAC ACTCACAAC 3960
ACCCGACCAC GGGCCCGAGC TACCGGGGGG GCATCGCCGC GGGCCCGGGA ACCAATTCTC 4020
CTGTCGGCGG GGGCGTCTT TGGATCC 4047

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 739 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

GGATCCAAAG GTCAAATCC CCACCTGGCA CTGTCCCCGG AGCGGGTCGC GCCCGGCCGG 60
CGCGCGGCCG GCGCTTGGC GCCAGAAGCG AGAGCCCTC GGGGCTCGCC CCCCAGCCTC 120
ACCGGGTCAG TGAACAAACG ATCAGAGTAG TGGTATTTCA CCGCGGCCCG GCAGGGCCGG 180
CGGACCCCGC CCGGGGCCCC TCGCGGGGAC ACCGGGGGGG CCGCGGGGGC CTCCCACTTA 240

```









GTACTCCTTG	CCAGGAATTC	AGTTGTAAT	AAAATTGAAC	CTGCTCAACA	GCTGAGGGAG	2580
ACTAGAAATG	ATGGGTCCAT	ATCCTGGTGC	ATTGTCTATC	AATTCAAACA	ATGGTGCAGC	2640
TACCAGCTTG	TAATTTTTAG	GGACTGCAAA	CAAGGCTTTT	TCTTGAAGCT	GAACCAAGAA	2700
CAACTTCTTA	TGTTCCCTAG	GCTTTGTAAT	ATGTGCAGGA	ATATATGGAT	ACTGAGGAGG	2760
TTCAAATTT	GGTCTCCACC	AGTTACCAAT	GCAATCGTCA	ATGACCCAGT	CTTGCAAAAC	2820
TCCATCCTGA	CGACCCAGTA	TCTCTGTCT	TAAGCGTTTT	AGTCCTTCAA	CTTCATCTTC	2880
TCCTGGGTTA	AGTTCAACAC	CAGGTAGTTT	GAAGAAAGTT	GTTCCAGCT	GCAGCAGTAA	2940
CACATGGGGT	AGCCGGTGCT	CATGTACAAT	CAGAACCCTT	TCTACAGTCC	TCCTCATTCC	3000
AATTTTATCA	AATTCTTCCC	TCATGCGCTG	AAATCTGGCT	GCAACAGAGC	TGTCCTTCTC	3060
GTAGAGGGGC	TCTTTTGTAC	CAAAAGTATA	ATTGGTAAGA	GGGTACAGGT	TGATGGTGCG	3120
CTCCAGGGTG	AGGGGCTTCG	TCTGCTGGAT	GTACTTGTG	CCGAAGTGA	TGACCCCGCG	3180
GGGCCAGCCG	GTCTGCGAGC	GATTGGGCGG	TACCACAGAC	ATGCTGGCGA	GCTCCGGCGC	3240
TGACGGCGAG	CAGAAAGTGG	CAGGCAGGGT	AGACTTTCCC	CGTGGCGGAA	GCCTCGTGCC	3300
GAATTC						3306

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4218 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAATTCGGCA	CGAGAATGGA	TCAACCTCAA	CAACACGTTA	AAGCTAGACG	AAAGAAGTAA	60
TACACAGTGT	ATGAGTCTCA	CATGAAATAC	CCGGATGTAA	ATCCAAAGAA	ACAGGAAGCA	120
GATTGGTGGT	TGCCAGGGAC	AAGGGCGGTG	GGAGGAGAAA	ATGGAGAGTA	ACGGGACTTT	180
ACTTTTGGAG	TGATGAGAAT	GTTTTGGAGC	TAGATAGAAG	TGGTGGTTGT	ACACCATTTG	240
GGATGTACTA	CCACTTAATT	GTTCACTTAA	AAAGTTAATT	TATGTGAATT	GCATCTTAAT	300
TAAAAACAAG	GATAACATTC	CAACTCCTGG	ACATTATCCT	TCCTTTCCAT	TTGATGTCAG	360
GCCCGTGTTA	GAATTCCTCAT	CCGGTTTGGT	CACTGCACCT	AAGATGTGGA	GAAATTAGGA	420
CGCACAGTTA	AGAGGAAGGA	TAACACTGAT	TAAGGTAGTG	CTTTTCTAGG	TTTCCCTTAA	480
ACAATTTAAC	AGATGGATAG	TGGCACCACT	TACGAGATGG	AAAAACCAGC	GGAAGGAAGA	540
TTTGGGGGAG	AAGTTAAGTT	TGTCTTGGGC	CTGTGTTTTG	CAACCTGAGT	GTAAAAGACA	600
TATGTTAAGT	CTTCAGTGGC	GAACACTAA	AACTAGAAAT	GGATCAGAAT	TTTATCTTTG	660
GATGTGACTT	CTCAAGGATG	GTCTTGTCC	TTCAGTGCCT	GGTCAAAATGA	CAAGATGGGC	720
AATCTTTTCC	TGAAGGTCCA	AGCACCTGAA	CGTGGCAGGG	TGACCCGATT	CCGATTTGCT	780
TAGAACAATC	CTAGTTTCATG	CCTATTGTCC	CTCATGTAAT	TAATATCACT	CTCAAAATGT	840
CTCATTTTGT	GCAATAAATT	CTGCAACGTG	ATGGCGCGAC	TCTCGCGGCC	CGAGCGGGCG	900
GACCTTGTCT	TCGAGGAAGA	GGACCTCCCC	TATGAGGAGG	AAATCATGCG	GAACCAATTC	960
TCTGTCAAAT	GCTGGCTTCA	CTACATCGAG	TTCAAACAGG	GCGCCCCGAA	GCCCGAGCTC	1020
AATCAGCTAT	ACGAGCGGGC	ACTCAAGCTG	CTGCCCTGCA	GCTACAAACT	CTGGTACCGA	1080
TACCTGAAGG	CGCGTCGGGC	ACAGGTGAAG	CATCGCTGTG	TGACCGACCC	TGCTATGAA	1140
GATGTCAACA	ACTGTCATGA	GAGGGCCTTT	GTGTTTCATG	ACAAGATGCC	TCGTCTGTGG	1200
CTAGATTACT	GCCAGTTCTT	CATGGACCCG	GGGCGCGTCA	CACACACCCG	CCGCACCTTC	1260
GACCGTGGCC	TCCGGGCAC	GCCATCACG	CAGCACTCTC	GAATTTGGCC	CCTGTATCTG	1320
CGCTTCTGCG	GCTCACACCC	ACTGCCTGAG	ACAGCTGTGC	GAGGCTATCG	GCGCTTCCTC	1380
AAGCTGAGTC	CTGAGAGTGC	AGAGGAGTAC	ATTGAGTACC	TCAAGTCAAG	TGACCGGCTG	1440
GATGAGGCGG	CCCAGCGCCT	GGCCACCGTG	GTGAACGACG	AGCGTTTCGT	GTCTAAGGCC	1500
GGCAAGTCCA	ACTACCAAGT	GTGGCAGGAG	CTGTGCGACC	TCATCTCCCA	GAATCCGGAC	1560
AAGGTACAGT	CCCTCAATGT	GGACGCCATC	ATCCGCGGGG	GCCTCACCCG	CTTCACCGAC	1620
CAGCTGGGCA	AGCTCTGGTG	TTCTCTCGCC	GACTACTACA	TCCGACGCGG	CCATTTCGAG	1680
AAGGCTCGGG	ACGTGTACGA	GGAGGCCATC	CGGACAGTGA	TGACCGTGCG	GGACTTCACA	1740
CAGGTGTTTG	ACAGCTACGC	CCAGTTTCGAG	GAGAGCATGA	TCGCTGCAAA	GATGGAGACC	1800
GCCTCGGAGC	TGGGGCGCGA	GGAGGAGGAT	GATGTGGACC	TGGAGCTGCG	CCTGGCCCGC	1860
TTGAGGACGC	TCATCAGCCG	GCGGCCCTTG	CTCCTCAACA	GCGTCTTGCT	GCGCCAAAAC	1920
CCACACCAAG	TGCACGAGTG	GCACAAGCGT	GTGCGCCTGC	ACCAGGGCCG	CCCCCGGGAG	1980
ATCATCAACA	CCTACACAGA	GGCTGTGCAG	ACGGTGGACC	CCTTCAAGGC	CACAGGCAAG	2040
CCCCACACTC	TGTGGGTGGC	GTTTGCCAAG	TTTTATGAGG	ACAACGGACA	GCTGGACGAT	2100
GCCCGTGTCA	TCCTGGAGAA	GGCCACCAAG	GTGAACCTTA	AGCAGGTGGA	TGACCTGGCA	2160
AGCGTGTGGT	GTCAGTGCAG	AGAGCTGGAG	CTCCGACACG	AGAACTACGA	TGAGGCCTTG	2220
GCGGTGCTGC	GAAAGGCCAC	GCGCTGCCT	GCCCGCCGGG	CCGAGTACTT	TGATGGTTCA	2280
GAGCCCGTGC	AGAACCGCGT	GTACAAGTCA	CTGAAGGTCT	GGTCCATGCT	GCGCGACCTG	2340
GAGGAGAGCC	TCGGCACCTT	CCAGTCCACC	AAGGCCGTGT	ACGACCGCAT	CCTGGACCTG	2400
CGTATCGCAA	CACCCAGAT	CGTCATCAAC	TATGCCATGT	TCCTGGAGGA	GCACAAGTAC	2460
TTGCGAGGAG	GCTTCAAGGC	GTACGAGCGC	GGCATCTCGC	TGTTCAAGTG	GCCCAACGTG	2520
TCCGACATGT	GGAGCACCTA	CCTGACCAAA	TTCATTGCCC	GCTATGGGGG	CCGCAAGCTG	2580
GAGCGGGCAC	GGGACCTGTT	TGAACAGGCT	CTGGACGGCT	GCCCCCAAAA	ATATGCCAAG	2640
ACCTTGTACC	TGCTGTACGC	ACAGCTGGAG	GAGGAGTGGG	GCCTGGCCCG	GCATGCCATG	2700
GCCGTGTACG	AGCGTGCCAC	CAGGGCCGTG	GAGCCCGCCC	AGCATATGA	CATGTTCAAC	2760

ATCTACATCA	AGCGGGCGGC	CGAGATCTAT	GGGGTCACCC	ACACCCGCGG	CATCTACCAG	2820
AAGGCCATTG	AGGTGCTGTC	GGACGAGCAC	GCGCGTGAGA	TGTGCCTGCG	GTTTGCAGAC	2880
ATGGAGTGCA	AGCTCGGGGA	GATTGACCGC	GCCCGGGCCA	TCTACAGCTT	CTGCTCCCAG	2940
ATCTGTGACC	CCCGGACGAC	CGCGCGTTTC	TGGCAGACGT	GGAAGGACTT	TGAGGTCCGG	3000
CATGGCAATG	AGGACACCAT	CAAGGAAATG	CTGCGTATCC	GGCGCAGCGT	GCAGGCCACG	3060
TACAACACGC	AGGTCAACTT	CATGGCCTCG	CAGATGCTCA	AGGTCTCGGG	CAGTGCCACG	3120
GGCACCCTGT	CTGACCTGGC	CCCTGGGCAG	AGTGGCATGG	ACGACATGAA	GCTGCTGGAA	3180
CAGCGGGCAG	AGCAGCTGGC	GGCTGAGGCG	GAGCGTGACC	AGCCCTTGCG	CGCCAGAGC	3240
AAGATCCTGT	TCGTGAGGAG	TGACGCCTCC	CGGGAGGAGC	TGGCAGAGCT	GGCACAGCAG	3300
GTCAACCCCG	AGGAGATCCA	GCTGGGCAG	GACGAGGACG	AGGACGAGAT	GGACCTGGAG	3360
CCCAACGAGG	TTCGGCTGGA	GCAGCAGAGC	GTGCCAGCCG	CAGTGTTTGG	GAGCCTGAAG	3420
GAAGACTGAC	CCGTCCCCTC	GTGCCGAATT	CGGCACGAGC	AAGACCAGCC	CCCAGATCAT	3480
TTGCCTCAAA	GGTTTTCCCT	CGAAGTCACA	AATGTTTCAA	GGAATCTCAA	ATTTTACAAA	3540
GTTTGAAGTG	TGGGCATTGG	TGGCCTGTGG	CTGTGTCCTC	TCTCTGTAGC	TGTTTTCTCC	3600
CTACATCCCT	GAAAGGAAGT	TGAGCCTGCT	CCTCCATCCG	CAGACCTCCC	TTTCCAGCGC	3660
CCAGGGCATG	GGGTGCTGTG	AGGGCAGCAT	GCTAGGTGTG	ACCGTGCTCC	TGGCCTCCAG	3720
GCCCGTGTC	CTCTGTCTCT	TAGCCCACTA	AGGCCCTGGC	CCATTGTGTC	TAAACAGGCA	3780
GTCGGACCTA	GAAAGAGCAG	ACAATCTCTC	TGGGTACCA	GTCTGGCTAG	GAGCTGGTCT	3840
CCTGACTGGG	ATCCAGGCCT	TCTCCCCTGC	CCATGTGAAT	TCCCAGGGGC	AGAGCCTGAA	3900
ATGTTGAACA	CAGCACTGGC	CAAGAGATG	TCACCGTGGG	AACCGAGGCT	CTCTTCTCCT	3960
CCTGCCTGCT	TTGCTGGGTT	CAGAGTAGCT	GAGGCTTGTC	TGAGAGGAGT	TGGAGTGCTG	4020
GTTTTCAACC	TGGTTGGTGT	GCTTTGCTTT	GAGGGCACTT	AGAAAGCCCA	GCCCAGCCCT	4080
TGCTCCTGCC	CTGCACACAG	CGGAGCGACT	TTTCTAGGTA	TGCTCTTGAT	TTCTGCAGAA	4140
GCAGCAGGTG	GCATGGAGCC	AAGAGGAAGT	GTGACTGAAA	CTGTCCACTC	ATAGCCCGGC	4200
TGCCGTATTG	AGAGGGCT					4218

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1187 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAGCTCGCGC	GCCTGCAGGT	CGACACTAGT	GGATCCAAAG	AATTCGGCAC	GAGGGAAACT	60
CAACGGTGTA	CGAGTGGAGG	ACAGGGACAG	AGCCCTCTGT	GGTGGAAACGA	CCCCACCTCG	120
AGGAGCTTCC	TGAGCAGGTG	GCAGAAGATG	CGATTGACTG	GGGCGACTTT	GGGGTAGAGG	180
CAGTGTCTGA	GGGGACTGAC	TCTGGCATCT	CTGCCGAGGC	TGCTGGAATC	GACTGGGGCA	240
TCTTCCCGGA	ATCAGATTCA	AAGGATCCTG	GAGGTGATGG	GATAGACTGG	GGAGACGATG	300
CTGTTGCTTT	GCAGATCACA	GTGCTGGAAG	CAGGAACCCA	GGCTCCAGAA	GGTGTTGCCA	360
GGGGCCGAGA	TGCCCTGACA	CTGCTTGAAT	ACACTGAGAC	CCGGAATCAG	TTCTTGATG	420
AGCTCATGGA	GCTTGAGATC	TTCTTAGCCC	AGAGAGCAGT	GGAGTTGAGT	GAGGAGGCAG	480
ATGTCCTGTC	TGTGAGCCAG	TTCCAGCTGG	CTCCAGCCAT	CCTGCAGGGC	CAGACCAAAG	540
AGAAGATGGT	TACCATGGTG	TCAGTGCTGG	AGGATCTGAT	TGGCAAGCTT	ACCAGTCTTC	600
AGCTGCAACA	CCTGTTTATG	ATCCTGGCCT	CACCAAGGTA	TGTGGACCGA	GTGACTGAAT	660
TCCTCCAGCA	AAAGCTGAAG	CAGTCCCAGC	TGCTGGCTTT	GAAGAAAAGG	CTGATGGTGC	720
AGAAGCAGCA	GGAGGCACTT	GAGGAGCAGG	CGGCTCTGGA	GCCTAAGCTG	GACCTGTAC	780
TGGAGAAGAT	CAAGGAGCTG	CAGAAGCTGA	TTGAAGCTGA	CATCTCCAAG	AGGTACAGCG	840
GGCGCCCTGT	GAACCTGATG	GGAACCTCTC	TGTGACACCC	TCCGTGTTCT	TGCCTGCCCCA	900
TCTTCTCCGC	TTTTGGGATG	AAGATGATAG	CCAGGGCTGT	TGTTTTGGGG	CCCTTCAAGG	960
CAAAAGACCA	GGCTGACTGG	AAGATGGAAA	GCCACAGGAA	GGAAGCGGCA	CCTGATGGTG	1020
ATCTTGGCAC	TCTCCATGTT	CTCTACAAGA	AGCTGTGGTG	ATTGGCCCTG	TGGTCTATCA	1080
GGCGAAAACC	ACAGATTCTC	CTTCTAGTTA	GTATAGCGCA	AAAAGCTTCT	CGAGAGTACT	1140
TCTAGAGCGG	CCGCGGGCCC	ATCGATTTC	CACCCGGGTG	GGGTACC		1187

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3306 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCCTCACTAA	AGGGAACAAA	AGCTGGAGCT	CGCGCGCCTG	CAGGTCGACA	CTAGTGGATC	60
GAAAGTTCGT	TACGCCAAGC	TCGAAATTAA	CTCTGGGCTG	ACCCATAAAC	ATTTGTCTGA	120

TCTAGGATAT	AGTTGCGTTT	CTTGCGGGCA	GCAATCTGGA	TGAGGCGGTT	GAGGCACTGG	180
GTGGCCTGCT	GGATCAGGAC	ATCCCAGCGG	CCAGCATAGT	TCCGCTGCCG	GCGTAGGCCG	240
ATCACCCGCA	TCTTATCCAT	GATGGCATTG	GTACCCAGGA	TGTTGTACTT	CTTGGGAAGGG	300
TTGGAGGCTG	CATGTTTGAT	GGCCCATGTG	GTCTTGCCAG	CAGCAGGCAG	GCCCACCATC	360
ATCAGAATCT	CACATTCTGC	CTTGCTCTTT	GGTCCAACGG	TGCCCCGGAT	ACGCTCACTA	420
AGGGGAAGGT	GCTGGATGAA	GGTAAACCCC	GGGAGGACAG	AACAGTAGGG	CTCTGCTCTC	480
TGTCCGAAGT	TGAACTCCAC	TGCGCAATTC	TTACCCAGGA	CATGAGGATA	GAGGGCCTGA	540
CCCCCAAGG	CTTCCTTCTG	GATTGCGAAA	GCAATGCCCA	TCCACTTTCC	ATTCTTGTTA	600
AAAGACAGTT	CCACGTCAAT	TCCACATTCA	AAATCCGCAA	AGCAGCCAAT	CACCGGAGAG	660
CTCTGCGGTG	CTAGGAGAGC	GGCTGGGCCC	GCAGACTGGG	GGGAAAGCTC	CGCAGCCGCA	720
GTGGGCCCCA	GGATCAGGCC	CCGCGTGGCC	TGGAGAAGCC	CAGTCTGGGC	TGGAGCGGGA	780
GCTGGACAGT	GTGGCCTTGC	GTTGCGCCCC	GGGAGCGCTG	CGAGTGTGCG	GGCCTCGGGT	840
GGATTGCTG	AGCACCAATA	CCTCACGGTT	GCCAACCTGG	GGTTTTAGCT	CCCTTGTTT	900
TAATCCCTTA	GGGGCGGGTG	GGGGCACGGG	AGGAAGGATG	GGCCAGCTGG	GTGCAATCCT	960
GCTGTAAGCC	AGCCATTCTT	TGATTCTTA	GAATTAACCT	AACGGTCGCG	CCGGAGGCCG	1020
CGGGGGCCGG	AGCGGAGCAG	CCGCGGCTGA	GGTTCCCGAG	TGCGCCGCTC	GGGGCTGCGC	1080
TCCGCGCGCG	GGACCCCGGC	CTCTGGCCGC	GCCGGCTCCG	GCCTCCGGGG	GGGCCGGGGC	1140
CGCCGGGACA	TGGTGCCAGT	CGCACCCCTT	CCCCGCCGCC	GCTGAGCTCG	CCGGCCGCGC	1200
CCGGGCTGGG	ACGTCCGAGC	GGGAAGATGT	TTTCCGCCCT	GAAGAAGCTG	GTGGGGTCGG	1260
ACCAGGCCCC	GGGCGGGGAC	AAGAACATCC	CCGCCGGGCT	GCAGTCCATG	AACCAGGCGT	1320
TGCAGAGGGC	CTTCGCCAAG	GGGGTGCACT	ACAACATGAA	GATAGTGATC	CGGGGAGACA	1380
GGAACACGGG	CAAGACAGCG	CTGTGGCACC	GCCTGCAGGG	CCGGCCGTTT	GTGGAGGAGT	1440
ACATCCCCAC	ACAGGAGATC	CAGGTCACTG	GCATCCACTG	GAGCTACAAG	ACCACGGATG	1500
ACATCGTGAA	GGTTGAAGTC	TGGGATGTAG	TAGACAAAGG	AAAATGCAAA	AAGCGAGGGC	1560
ACGGCTTAAA	GATGGAGAAC	GACCCCAAGG	AGNCGGAGTC	TGAAATGGCC	CTGGATGCTG	1620
AGTTCTCTGA	CGTGTACAAG	AACTGCAACG	GGGTGGTCAT	GATGTTTCGAC	ATTACCAAGC	1680
AGTGGACCTT	CAATTACATT	CTCCGGGAGC	TTCCAAAAGT	GCCCACCCAC	GTGCCAGTGT	1740
GGGTGCTGGG	GAATACCGG	GACATGGGCG	AGCACCGAGT	CATCTCGCCG	GACGACGTGC	1800
GTGACTTCAT	CGACAACCTG	CAGGACCTTC	CAGGTTCTCT	CTACTTCCGC	TATGCTGAGT	1860
CTTCCATGAA	GAACAGCTTC	GGCCTAAAGT	ACCTTCATAA	GTTCTTCAAT	ATCCCATTTT	1920
TGCAGCTTCA	GAGGGAGACG	CTGTTGCGGC	AGCTGGAGAC	GAACCAAGCT	GACATGGACG	1980
CCAGCTGGGA	GGAGCTGTGG	GTGCAGCAGG	AGACGGAGGA	CCAGAACTAC	GGCATCTTCC	2040
TGGAGATGAT	GGAGGCTCGC	AGCCGTGGCC	ATGCGTCCCC	ACTGGCGGCG	AACGGGCAGA	2100
GCCCCATCCC	GGGCTCCGAG	TACCCAGTCC	TGCTGCAACC	CGCTGTGTCC	ACGGGGAGCT	2160
CCAGCCCCGG	CACACCCGAG	CCCGCCCCAC	AGCTGCCCTT	CAATGCTGCC	CCACCATCCT	2220
CTGTGCCCCG	TGTACCAACC	TCAGAGGCCC	TGCCCCCAAC	TGCGTGCCCC	TCAGCCCCCG	2280
CCCCACGGCG	CAGCATCATC	TCTAGGCTGT	TTGGGACGTC	ACCTGCCACC	GAGGCAGCCC	2340
CTCCACCTCC	AGAGCCAGTC	CCGGCCGCGC	AGGGCCCAAG	AACGGTCCAG	AGTGTGGAGG	2400
ACTTTGTTCC	TGACGACCGC	CTGGACCCTG	GCTTCTGGA	AGACACAACC	CCGCCAGGGG	2460
ACGAGAAGAA	GGTGGGGGCC	AAGGCTGCCC	AGCAGGACAG	TGACAGTGAT	GGGGAGGGCC	2520
TGGGCGGCAA	CCCAGTGGTG	GCAGGGTTCC	AGGACGATGT	GGACCTCGAA	GACCAAGCCAC	2580
GTGGGAGTCC	CCCCTGCTCT	GCAGGCCCCG	TCCCCAGTCA	AGACATCACT	CTTTCGAGTG	2640
AGGAGGAAGC	AGAAGTGGCA	GCTCCCAACA	AAGGCCCTGC	CCCAGCTCCC	CAGCAGTGCT	2700
CAGAGCCAGA	GACCAAGTGG	TCTCCATAC	CAGCTTCGAA	GCCACGGAGG	GGGACAGCTC	2760
CCACGAGGAC	CGCAGCACCC	CCCTGGCCAG	GCGGTGTCTC	TGTTCCGACA	GGTCCGGAGA	2820
AGCGCAGCAG	CACCAAGGCC	CCTGCTGAGA	TGGAGCCGGG	GAAGGGTGAG	CAGGCCTCCT	2880
CGTCGGAGAG	TGACCCCGAG	GGACCCATTG	CTGCACAAAT	GCTGTCTTTC	GTGATGGATG	2940
ACCCCGACTT	TGAGAGCGAG	GGATCAGACA	CACAGCGCAG	GGCGGATGAC	TTTCCCGTGC	3000
GAGATGACCC	CTCCGATGTG	ACTGACGAGG	ATGAGGGCCC	TGCCGAGCCG	CCCCACCCCC	3060
CCAAGCTCCC	TCTCCCCGCC	TTCAGACTGA	AGAATGACTC	GGACCTCTTC	GGGCTGGGGC	3120
TGGAGGAGGC	CGGACCCAAG	GAGAGCAGTG	AGGAAGGTAA	GGAGGGCAAA	ACCCCTCTTA	3180
AGGAGAAGAA	AAAAAAAACA	AAAAGCTTCT	CGAGAGTACT	TCTAGAGCGG	CCGCGGGCCC	3240
ATCGATTTTC	CACCCGGGTG	GGGTACCAGG	TAAGTGATAC	CAATTCGCCC	TATAGTGAGT	3300
CGTATT						3306

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGCGGGGCCA GAGTGGGCTG

20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid



(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCAGTCCTGG CCTGCGGATG

20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTCGACAGGA GAATTGGTTC

20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCCTGGGTTC GGTGCGGGAC

20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGGTCGGGTG TTTGTGAGTG

20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCTCTTCCGT CTCCTCAGTG

20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGATTGCTAG TCTCACAGAC

20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTAAGGGTGG CTGAAGGGAC

20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ACCTTCCTC CCTGTCACAG

20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TGGTCGGGTG TTTGTGAGTG

20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ACACCATTC AGAAATTCAG

20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AAACTGCAGG TGGCTGAGTC

20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTCCTAATGT TTTCAGGGAG

20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AAAACCTATG GTTACAATTC

20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCCTAGACAT GGTTC AAGTG

20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATATAATTA GTTCTCCATC

20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATGCCTGTTT CAGGCTGCAC

20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGACGGCGAC CTCCACCCAC

20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGGCTCCTCC GACGCCTGAG

20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGTCTAGCCC TGGCCTTGAC

20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTCACGGGG ACTCCGGCAG

20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CAGCTTTCCC TGGGCACATG

20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CACAGCTGTC TCAAGCCCAG

20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACTGTTCCCC CTACATGATG

20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATCATATCCT CTTGCTGGTC

20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTTCCAGAG CTTGTCTGTG

20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTTTGGCAGA CTCATAGTTG

20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TAGCAGGGAG CCATGACCTG

20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CTTGCGCCA GAAGCGAGAG

20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CCTCTCTCTC TCTCTCTC

20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCCCCGCTGA TTCCGCCAAG

20

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CTTTTGAAT TCGGCACGAG

20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CCCCTGGTCC GCACCAGTTC

20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GAGAAGGGTC GGGGCGGCAG

20

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AAATCACATC GCGTCAACAC

20

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TAAGAGAGTC ATAGTTACTC

20

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GCTCTAGAAG TACTCTCGAG

20

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ACTCTGGCCA TCAGGAGATC

20

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CAGGCGTTGT AGATGTTCTG

20

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

AGTGGCAGGC AGAAGTAATG

20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(A) LENGTH: 20 base pairs



(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TGGGTGATGC CTTTGCTGAC

20

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

AAAACAAGAT CAAGGTGATG

20

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TTGCCACAT TGCTATGGTG

20

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GACCAAGATC AGAAGTAGAG

20

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CCCCTGGGCC AATGATGTTG

20

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TCTTCCACC ATAGCAATG

19

[illegible][illegible]

- [illegible]

[illegible][illegible][illegible][illegible][illegible]

- [illegible]

[illegible][illegible][illegible][illegible][illegible]

- [illegible]

[illegible][illegible][illegible][illegible][illegible]

- [illegible]

[illegible][illegible][illegible][illegible][illegible]

- [illegible]

[illegible][illegible][illegible][illegible][illegible]

- [illegible]

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GGAAATGAGG TGGGGCGATC

20

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CTTTGCCTTG GACAAGGATG

20

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GCACCTGCCA TTGGGGGTAG

20

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GGTGAAGCC ATTGACGGTG

20

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

TGCGTCTCTC GTCGCTGCTG

20

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GCGGAACTC TGTGGTGCTG

20

(2) INFORMATION FOR SEQ ID NO:74:



20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

TCGAGGTTCA GAGCGTAGTG

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

TCTTGGATCT CTGGCACCTC

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

CCATCAGAGT GAAGGAGGAG

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

CCATCTTCCA CTGGTCAGAG

20.

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

CTCCTTCTCT TGGATCTCTG

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid

[illegible]

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TTACTTCAGC ACTGTTAGTC

20

(2) INFORMATION FOR SEQ ID NO:86:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

AGGGAGGTAG CTCAAAGCTC

20

(2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TGGGTCCACA GTTCGCACAG

20

(2) INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

CAACTCTGTG ATGGCTCCAG

20

(2) INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

AGCAGGGTTC TGTCAAGAC

20

(2) INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

CCATTGGGTG CTAGTCTCTC

20

(2) INFORMATION FOR SEQ ID NO:91:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CAGCCATGCT GTCCCAGCAG

20

(2) INFORMATION FOR SEQ ID NO:92:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

CTGGACCTGA GGTAGCGCTG

20

(2) INFORMATION FOR SEQ ID NO:93:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

ATAACCACCC TGAGGCACTG

20

(2) INFORMATION FOR SEQ ID NO:94:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

CCTGCAGGTC GACACTAGTG

20

(2) INFORMATION FOR SEQ ID NO:95:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

AATTGGAATG AGGAGGACTG

20

(2) INFORMATION FOR SEQ ID NO:96:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GCTCTAGAAG TACTCTCGAG

20

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

ATTGTATGAC AATGCACCAG

20

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

TCCACAGAGG GCTTCATCAC

20

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

CCTGACTGGC CTAAGCACAG

20

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

AAGCCTCATA ACCACCAGTG

20

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

TGTCACGGT GACAAGTGTG

20

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:



- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

TTGTACACCA GCTGCAGGTC

20

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

GGGTGTGGTG CAGATGAGTC

20

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

ATCACACTCT TATAGCTCAG

20

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

GTGGGAAGCT TTCCTCAGAC

20

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

TGATGAACAT GGGCCTGGAG

20

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:



[illegible][illegible]

20

[illegible][illegible][illegible][illegible]

20

[illegible][illegible][illegible][illegible]

20

[illegible][illegible][illegible][illegible]

20

[illegible][illegible][illegible][illegible]

20

[illegible][illegible][illegible][illegible]

20

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

TCTTGAGGGG ACTGACTCTG

20

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

TGAGTGAGGA GGCAGATGTC

20

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

TGGCTTTGAA GAAAGAGCTG

20

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

GCAAAAGACC AGGCTGACTG

20

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

TGCAGCTCCT TGGTCTTCTC

20

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

[illegible]

20

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(2) INFORMATION FOR SEQ ID NO:130:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs



(2) INFORMATION FOR SEQ ID NO:136:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

GTCACCACTG GTGCCTGCAG

20

(2) INFORMATION FOR SEQ ID NO:137:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

ACCTCACGGT TGCCAACCTG

20

(2) INFORMATION FOR SEQ ID NO:138:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

CGCAACAGCG TCTCCCTCTG

20

(2) INFORMATION FOR SEQ ID NO:139:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

AGTACCTTCA TAAGTTCTTC

20

(2) INFORMATION FOR SEQ ID NO:140:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

TCCCAGACTT CAACCTTCAC

20

(2) INFORMATION FOR SEQ ID NO:141:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

AAACATCTTC CCGGTCGGAC

20

(2) INFORMATION FOR SEQ ID NO:142:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

GCTGAGCACC TTACCTCAC

20

(2) INFORMATION FOR SEQ ID NO:143:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

GACGTCCGTC CGGGAAGATG

20

(2) INFORMATION FOR SEQ ID NO:144:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

ACACAGGAGA TGCAGGTCAC

20

(2) INFORMATION FOR SEQ ID NO:145:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

GAGTCTTCCA TGAAGAACAG

20

(2) INFORMATION FOR SEQ ID NO:146:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

GCAGTGAGGA AGGTAAGGAG

20

(2) INFORMATION FOR SEQ ID NO:147:



(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4047 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence  
(B) LOCATION: 378...1799  
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

```
GGATCCAAAG GACGCCCCCG CCGACAGGAG AATTGGTTCC CGGGCCCGCG GCGATGCCCC    60
CCCGGTAGCT CGGGCCCGTG GTCGGGTGTT TGTGAGTGTT TCTATGTGGG AGAAGGAGGA    120
GGAGGAGGAA GAAGAAGCAA CGATTGTCT TCTCGGCTGG TCTCCCCCG GCTCTACATG    180
TTCCCCGCAC TGAGGAGACG GAAGAGGAGC CGTAGCCGCC CCCCCTCCCG GCCCGGATTA    240
TAGTCTCTCG CCACAGCGGC CTCGCCCTCC CTTGGATTG AGACGCCGAT TCGCCCAGTG    300
TTTGGGAAAT GGGGAAGTAAT GACAGCTGGC ACCTGAAC TAAGTCTTTA TAGGCAACAC    360
CATTCCAGAA ATTCAGG ATG AAT GGG GAT ATG CCC CAT GTC CCC ATT ACT    410
               Met Asn Gly Asp Met Pro His Val Pro Ile Thr
                   1             5             10

ACT CTT GCG GGG ATT GCT AGT CTC ACA GAC CTC CTG AAC CAG CTG CCT    458
Thr Leu Ala Gly Ile Ala Ser Leu Thr Asp Leu Leu Asn Gln Leu Pro
                   15             20             25

CTT CCA TCT CCT TTA CCT GCT ACA ACT ACA AAG AGC CTT CTC TTT AAT    506
Leu Pro Ser Pro Leu Pro Ala Thr Thr Lys Ser Leu Leu Phe Asn
                   30             35             40

GCA CGA ATA GCA GAA GAG GTG AAC TGC CTT TTG GCT TGT AGG GAT GAC    554
Ala Arg Ile Ala Glu Glu Val Asn Cys Leu Leu Ala Cys Arg Asp Asp
                   45             50             55

AAT TTG GTT TCA CAG CTT GTC CAT AGC CTC AAC CAG GTA TCA ACA GAT    602
Asn Leu Val Ser Gln Leu Val His Ser Leu Asn Gln Val Ser Thr Asp
                   60             65             70             75

CAC ATA GAG TTG AAA GAT AAC CTT GGC AGT GAT GAC CCA GAA GGT GAC    650
His Ile Glu Leu Lys Asp Asn Leu Gly Ser Asp Asp Pro Glu Gly Asp
                   80             85             90

ATA CCA GTC TTG TTG CAG GCC GTC CTG GCA AGG AGT CCT AAT GTT TTC    698
Ile Pro Val Leu Leu Gln Ala Val Leu Ala Arg Ser Pro Asn Val Phe
                   95             100             105

AGG GAG AAA AGC ATG CAG AAC AGA TAT GTA CAA AGT GGA ATG ATG ATG    746
Arg Glu Lys Ser Met Gln Asn Arg Tyr Val Gln Ser Gly Met Met Met
                   110             115             120

TCT CAG TAT AAA CTT TCT CAG AAT TCC ATG CAC AGT AGT CCT GCA TCT    794
Ser Gln Tyr Lys Leu Ser Gln Asn Ser Met His Ser Ser Pro Ala Ser
                   125             130             135

TCC AAT TAT CAA CAA ACC ACT ATC TCA CAT AGC CCC TCC AGC CGG TTT    842
Ser Asn Tyr Gln Gln Thr Thr Ile Ser His Ser Pro Ser Ser Arg Phe
                   140             145             150             155

GTG CCA CCA CAG ACA AGC TCT GGG AAC AGA TTT ATG CCA CAG CAA AAT    890
Val Pro Pro Gln Thr Ser Ser Gly Asn Arg Phe Met Pro Gln Gln Asn
                   160             165             170

AGC CCA GTG CCT AGT CCA TAC GCC CCA CAA AGC CCT GCA GGA TAC ATG    938
Ser Pro Val Pro Ser Pro Tyr Ala Pro Gln Ser Pro Ala Gly Tyr Met
                   175             180             185

CCA TAT TCC CAT CCT TCA AGT TAC ACA ACA CAT CCA CAG ATG CAA CAA    986
Pro Tyr Ser His Pro Ser Ser Tyr Thr Thr His Pro Gln Met Gln Gln
                   190             195             200
```

[illegible]



Pro Ile Val Ala Gly Gly Leu Arg Asn Ile His Asp Asn Lys Val Ser  
 210 215 220  
 Gly Pro Leu Ser Gly Asn Ser Ala Asn His His Ala Asp Asn Pro Arg  
 225 230 235 240  
 His Gly Ser Ser Glu Asp Tyr Leu His Met Val His Arg Leu Ser Ser  
 245 250 255  
 Asp Asp Gly Asp Ser Ser Thr Met Arg Asn Ala Ala Ser Phe Pro Leu  
 260 265 270  
 Arg Ser Pro Gln Pro Val Cys Ser Pro Ala Gly Ser Glu Gly Thr Pro  
 275 280 285  
 Lys Gly Ser Arg Pro Pro Leu Ile Leu Gln Ser Gln Ser Leu Pro Cys  
 290 295 300  
 Ser Ser Pro Arg Asp Val Pro Pro Asp Ile Leu Leu Asp Ser Pro Glu  
 305 310 315 320  
 Arg Lys Gln Lys Lys Gln Lys Lys Met Lys Leu Gly Lys Asp Glu Lys  
 325 330 335  
 Glu Gln Ser Glu Lys Ala Ala Met Tyr Asp Ile Ile Ser Ser Pro Ser  
 340 345 350  
 Lys Asp Ser Thr Lys Leu Thr Leu Arg Leu Ser Arg Val Arg Ser Ser  
 355 360 365  
 Asp Met Asp Gln Gln Glu Asp Met Ile Ser Gly Val Glu Asn Ser Asn  
 370 375 380  
 Val Ser Glu Asn Asp Ile Pro Phe Asn Val Gln Tyr Pro Gly Gln Thr  
 385 390 395 400  
 Ser Lys Thr Pro Ile Thr Pro Gln Asp Ile Asn Arg Pro Leu Asn Ala  
 405 410 415  
 Ala Gln Cys Leu Ser Gln Gln Glu Gln Thr Ala Phe Leu Pro Ala Asn  
 420 425 430  
 Gln Val Pro Val Leu Gln Gln Asn Thr Ser Val Ala Ala Lys Gln Pro  
 435 440 445  
 Gln Thr Asn Ser His Lys Thr Leu Val Gln Pro Gly Thr Gly Ile Glu  
 450 455 460  
 Val Ser Ala Glu Leu Pro Lys Asp Lys Thr  
 465 470

(2) INFORMATION FOR SEQ ID NO:149:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2998 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 26...799
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

AAGCTTTTGG AATTCGGCAC GAGAT GCT ACA CAG GCT ATA TTT GAA ATA CTG	52
Ala Thr Gln Ala Ile Phe Glu Ile Leu	
1 5	
GAG AAA TCC TGG TTG CCC CAG AAT TGT ACA CTG GTT GAT ATG AAG ATT	100
Glu Lys Ser Trp Leu Pro Gln Asn Cys Thr Leu Val Asp Met Lys Ile	
10 15 20 25	
GAA TTT GGT GTT GAT GTA ACC ACC AAA GAA ATT GTT CTT GCT GAT GTT	148
Glu Phe Gly Val Asp Val Thr Thr Lys Glu Ile Val Leu Ala Asp Val	
30 35 40	
ATT GAC AAT GAT TCC TGG AGA CTC TGG CCA TCA GGA GAT CGA AGC CAA	196
Ile Asp Asn Asp Ser Trp Arg Leu Trp Pro Ser Gly Asp Arg Ser Gln	
45 50 55	
CAG AAA GAC AAA CAG TCT TAT CGG GAC CTC AAA GAA GTA ACT CCT GAA	244
Gln Lys Asp Lys Gln Ser Tyr Arg Asp Leu Lys Glu Val Thr Pro Glu	
60 65 70	
GGG CTC CAA ATG GTA AAG AAA AAC TTT GAG TGG GTT GCA GAG AGA GTA	292





Pro Lys Ala Gln Asn Lys Arg Lys Arg Cys Leu Phe Gly Gly Gln Glu  
 20 25 30  
 Pro Ala Pro Lys Glu Gln Pro Pro Leu Gln Pro Pro Gln Gln Ser  
 35 40 45  
 Ile Arg Val Lys Glu Glu Gln Tyr Leu Gly His Glu Gly Pro Gly Gly  
 50 55 60  
 Ala Val Ser Thr Ser Gln Pro Val Glu Leu Pro Pro Ser Ser Leu  
 65 70 75 80  
 Ala Leu Leu Asn Ser Val Val Tyr Gly Pro Glu Arg Thr Ser Ala Ala  
 85 90 95  
 Met Leu Ser Gln Gln Val Ala Ser Val Lys Trp Pro Asn Ser Val Met  
 100 105 110  
 Ala Pro Gly Arg Gly Pro Glu Arg Gly Gly Gly Gly Val Ser Asp  
 115 120 125  
 Ser Ser Trp Gln Gln Gln Pro Gly Gln Pro Pro Pro His Ser Thr Trp  
 130 135 140  
 Asn Cys His Ser Leu Ser Leu Tyr Ser Ala Thr Lys Gly Ser Pro His  
 145 150 155 160  
 Pro Gly Val Gly Val Pro Thr Tyr Tyr Asn His Pro Glu Ala Leu Lys  
 165 170 175  
 Arg Glu Lys Ala Gly Gly Pro Gln Leu Asp Arg Tyr Val Arg Pro Met  
 180 185 190  
 Met Pro Gln Lys Val Gln Leu Glu Val Gly Arg Pro Gln Ala Pro Leu  
 195 200 205  
 Asn Ser Phe His Ala Ala Lys Lys Pro Pro Asn Gln Ser Leu Pro Leu  
 210 215 220  
 Gln Pro Phe Gln Leu Ala Phe Gly His Gln Val Asn Arg Gln Val Phe  
 225 230 235 240  
 Arg Gln Gly Pro Pro Pro Pro Asn Pro Val Ala Ala Phe Pro Pro Gln  
 245 250 255  
 Lys Gln Gln Gln Gln Gln Pro Gln Gln Gln Gln Gln Gln Gln  
 260 265 270  
 Ala Ala Leu Pro Gln Met Pro Leu Phe Glu Asn Phe Tyr Ser Met Pro  
 275 280 285  
 Gln Gln Pro Ser Gln Gln Pro Gln Asp Phe Gly Leu Gln Pro Ala Gly  
 290 295 300  
 Pro Leu Gly Gln Ser His Leu Ala His His Ser Met Ala Pro Tyr Pro  
 305 310 315 320  
 Phe Pro Pro Asn Pro Asp Met Asn Pro Glu Leu Arg Lys Ala Leu Leu  
 325 330 335  
 Gln Asp Ser Ala Pro Gln Pro Ala Leu Pro Gln Val Gln Ile Pro Phe  
 340 345 350  
 Pro Arg Arg Ser Arg Arg Leu Ser Lys Glu Gly Ile Leu Pro Pro Ser  
 355 360 365  
 Ala Leu Asp Gly Ala Gly Thr Gln Pro Gly Gln Glu Ala Thr Gly Asn  
 370 375 380  
 Leu Phe Leu His His Trp Pro Leu Gln Gln Pro Pro Pro Gly Ser Leu  
 385 390 395 400  
 Gly Gln Pro His Pro Glu Ala Leu Gly Phe Pro Leu Glu Leu Arg Glu  
 405 410 415  
 Ser Gln Leu Leu Pro Asp Gly Glu Arg Leu Ala Pro Asn Gly Arg Glu  
 420 425 430  
 Arg Glu Ala Pro Ala Met Gly Ser Glu Glu Gly Met Arg Ala Val Ser  
 435 440 445  
 Thr Gly Asp Cys Gly Gln Val Leu Arg Gly Gly Val Ile Gln Ser Thr  
 450 455 460  
 Arg Arg Arg Arg Arg Ala Ser Gln Glu Ala Asn Leu Leu Thr Leu Ala  
 465 470 475 480  
 Gln Lys Ala Val Glu Leu Ala Ser Leu Gln Asn Ala Lys Asp Gly Ser  
 485 490 495  
 Gly Ser Glu Glu Lys Arg Lys Ser Val Leu Ala Ser Thr Thr Lys Cys  
 500 505 510  
 Gly Val Glu Phe Ser Glu Pro Ser Leu Ala Thr Lys Arg Ala Arg Glu  
 515 520 525  
 Asp Ser Gly Met Val Pro Leu Ile Ile Pro Val Ser Val Pro Val Arg  
 530 535 540  
 Thr Val Asp Pro Thr Glu Ala Ala Gln Ala Gly Gly Leu Asp Glu Asp  
 545 550 555 560  
 Gly Lys Gly Leu Glu Gln Asn Pro Ala Glu His Lys Pro Ser Val Ile  
 565 570 575  
 Val Thr Arg Arg Arg Ser Thr Arg Ile Pro Gly Thr Asp Ala Gln Ala  
 580 585 590  
 Gln Ala Glu Asp Met Asn Val Lys Leu Glu Gly Glu Pro Ser Val Arg











835  
Leu Lys Glu Asp  
850

840

845

(2) INFORMATION FOR SEQ ID NO:154:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 693 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

```
Met Phe Ser Ala Leu Lys Lys Leu Val Gly Ser Asp Gln Ala Pro Gly
 1      5      10      15
Arg Asp Lys Asn Ile Pro Ala Gly Leu Gln Ser Met Asn Gln Ala Leu
 20      25      30
Gln Arg Arg Phe Ala Lys Gly Val Gln Tyr Asn Met Lys Ile Val Ile
 35      40      45
Arg Gly Asp Arg Asn Thr Gly Lys Thr Ala Leu Trp His Arg Leu Gln
 50      55      60
Gly Arg Pro Phe Val Glu Glu Tyr Ile Pro Thr Gln Glu Ile Gln Val
 65      70      75      80
Thr Ser Ile His Trp Ser Tyr Lys Thr Thr Asp Asp Ile Val Lys Val
 85      90      95
Glu Val Trp Asp Val Val Asp Lys Gly Lys Cys Lys Lys Arg Gly Asp
100      105      110
Gly Leu Lys Met Glu Asn Asp Pro Gln Glu Xaa Glu Ser Glu Met Ala
115      120      125
Leu Asp Ala Glu Phe Leu Asp Val Tyr Lys Asn Cys Asn Gly Val Val
130      135      140
Met Met Phe Asp Ile Thr Lys Gln Trp Thr Phe Asn Tyr Ile Leu Arg
145      150      155      160
Glu Leu Pro Lys Val Pro Thr His Val Pro Val Cys Val Leu Gly Asn
165      170      175
Tyr Arg Asp Met Gly Glu His Arg Val Ile Leu Pro Asp Asp Val Arg
180      185      190
Asp Phe Ile Asp Asn Leu Asp Arg Pro Pro Gly Ser Ser Tyr Phe Arg
195      200      205
Tyr Ala Glu Ser Ser Met Lys Asn Ser Phe Gly Leu Lys Tyr Leu His
210      215      220
Lys Phe Phe Asn Ile Pro Phe Leu Gln Leu Gln Arg Glu Thr Leu Leu
225      230      235      240
Arg Gln Leu Glu Thr Asn Gln Leu Asp Met Asp Ala Thr Leu Glu Glu
245      250      255
Leu Ser Val Gln Gln Glu Thr Glu Asp Gln Asn Tyr Gly Ile Phe Leu
260      265      270
Glu Met Met Glu Ala Arg Ser Arg Gly His Ala Ser Pro Leu Ala Ala
275      280      285
Asn Gly Gln Ser Pro Ser Pro Gly Ser Gln Ser Pro Val Leu Pro Ala
290      295      300
Pro Ala Val Ser Thr Gly Ser Ser Ser Pro Gly Thr Pro Gln Pro Ala
305      310      315      320
Pro Gln Leu Pro Leu Asn Ala Ala Pro Pro Ser Ser Val Pro Pro Val
325      330      335
Pro Pro Ser Glu Ala Leu Pro Pro Pro Ala Cys Pro Ser Ala Pro Ala
340      345      350
Pro Arg Arg Ser Ile Ile Ser Arg Leu Phe Gly Thr Ser Pro Ala Thr
355      360      365
Glu Ala Ala Pro Pro Pro Pro Glu Pro Val Pro Ala Ala Gln Gly Pro
370      375      380
Ala Thr Val Gln Ser Val Glu Asp Phe Val Pro Asp Asp Arg Leu Asp
385      390      395      400
Arg Ser Phe Leu Glu Asp Thr Thr Pro Ala Arg Asp Glu Lys Lys Val
405      410      415
Gly Ala Lys Ala Ala Gln Gln Asp Ser Asp Ser Asp Gly Glu Ala Leu
420      425      430
Gly Gly Asn Pro Met Val Ala Gly Phe Gln Asp Asp Val Asp Leu Glu
435      440      445
Asp Gln Pro Arg Gly Ser Pro Pro Leu Pro Ala Gly Pro Val Pro Ser
450      455      460
```

Gln Asp Ile Thr Leu Ser Ser Glu Glu Glu Ala Glu Val Ala Ala Pro  
 465 470 475 480  
 Thr Lys Gly Pro Ala Pro Ala Pro Gln Gln Cys Ser Glu Pro Glu Thr  
 485 490 495  
 Lys Trp Ser Ser Ile Pro Ala Ser Lys Pro Arg Arg Gly Thr Ala Pro  
 500 505 510  
 Thr Arg Thr Ala Ala Pro Pro Trp Pro Gly Gly Val Ser Val Arg Thr  
 515 520 525  
 Gly Pro Glu Lys Arg Ser Ser Thr Arg Pro Pro Ala Glu Met Glu Pro  
 530 535 540  
 Gly Lys Gly Glu Gln Ala Ser Ser Ser Glu Ser Asp Pro Glu Gly Pro  
 545 550 555 560  
 Ile Ala Ala Gln Met Leu Ser Phe Val Met Asp Asp Pro Asp Phe Glu  
 565 570 575  
 Ser Glu Gly Ser Asp Thr Gln Arg Arg Ala Asp Asp Phe Pro Val Arg  
 580 585 590  
 Asp Asp Pro Ser Asp Val Thr Asp Glu Asp Glu Gly Pro Ala Glu Pro  
 595 600 605  
 Pro Pro Pro Pro Lys Leu Pro Leu Pro Ala Phe Arg Leu Lys Asn Asp  
 610 615 620  
 Ser Asp Leu Phe Gly Leu Gly Leu Glu Glu Ala Gly Pro Lys Glu Ser  
 625 630 635 640  
 Ser Glu Glu Gly Lys Glu Gly Lys Thr Pro Ser Lys Glu Lys Lys Lys  
 645 650 655  
 Lys Thr Lys Ser Phe Ser Arg Val Leu Leu Glu Arg Pro Arg Ala His  
 660 665 670  
 Arg Phe Ser Thr Arg Val Gly Tyr Gln Val Ser Val Pro Asn Ser Pro  
 675 680 685  
 Tyr Ser Glu Ser Tyr  
 690

## CLAIMS

What is claimed as the invention is:

1. An isolated polynucleotide comprising a nucleotide sequence with the following properties:
  - a) the sequence is expressed at the mRNA level in Jurkat T cells;
  - b) when COS-1 cells expressing TNF receptor are genetically altered to express the sequence, the cells have increased enzymatic activity for cleaving and releasing the receptor.
2. The polynucleotide of claim 1, wherein the nucleotide sequence is contained in a sequence selected from the group consisting of
  - a) SEQ. ID NO:1;
  - b) SEQ. ID NO:2 or SEQ. ID NO:3;
  - c) SEQ. ID NO:4;
  - d) SEQ. ID NO:5;
  - e) SEQ. ID NO:6;
  - f) SEQ. ID NO:7;
  - g) SEQ. ID NO:8;
  - h) SEQ. ID NO:9; and
  - i) SEQ. ID NO:10.
3. An isolated polynucleotide comprising at least 30 consecutive nucleotides in said nucleotide sequence of a polynucleotide according to any of claims 1-3
4. An isolated polynucleotide comprising a linear sequence of at least 50 consecutive nucleotides at least 90% identical to a sequence contained in said nucleotide sequence of the polynucleotide of claim 1.

5. An isolated polynucleotide of at least 50 nucleotides capable of hybridizing specifically to said nucleotide sequence of a polynucleotide according to any of claims 1-3 at 68°C in 0.5 M phosphate buffer pH 7, 7% SDS, and 100 µg/mL salmon sperm DNA, followed by washing in a buffer containing 3X SSC.
6. An antisense polynucleotide or ribozyme comprising at least 10 consecutive nucleotides in said nucleotide sequence of a polynucleotide according to claim 1 or 2, which inhibits the expression of a TRRE modulator.
7. An isolated polypeptide comprising an amino acid sequence encoded by a polynucleotide according to any of claims 1-5.
8. The polypeptide of claim 7, selected from the group consisting of SEQ. ID NOS: 147-158.
9. An isolated polypeptide, comprising at least 10 consecutive residues in said amino acid sequence of a polypeptide according to claim 7 or 8.
10. An isolated polypeptide, comprising at least 15 consecutive amino acids which are at least 80% identical to a sequence contained in said amino acid sequence of the polypeptide according to claim 7 or 8.
11. The polypeptide of claim 7-11, which when incubated with COS-1 cells expressing TNF receptor, promotes enzymatic cleavage and release of the receptor.
12. The polypeptide of claims 7-11, which either:
  - a) lacks a membrane spanning sequence; or





19. An assay method for determining altered expression of a modulator of TRRE activity in a cell or tissue sample, comprising the steps of:
  - a) contacting the sample with the antibody of claim 16 under conditions that permit the antibody to bind the modulator if present in the sample, thereby forming an antibody-antigen complex; and
  - b) determining complex formed in step a), as a measure of the modulator.
20. A method for assessing a disease condition associated with altered TRRE activity in a subject, comprising determining altered TRRE activity in the sample from the subject according to claim 18, or determining altered expression of a TRRE modulator according to claim 19, and then correlating the extent of alteration with the disease condition.
21. A method for decreasing signal transduction from a cytokine into a cell, comprising contacting the cell with a polypeptide according to any of claims 7-8 and 11-12, or with a polynucleotide according to any of claims 1-3 and 15.
22. A method for increasing signal transduction from a cytokine into a cell, comprising contacting the cell with a polynucleotide according to claim 6, or with an antibody according to claim 16.
23. The method according to claim 21 or claim 22, wherein the cytokine is TNF.
24. A method for screening polynucleotides for an ability to modulate TRRE activity, comprising the steps of:
  - a) providing cells that express both TRRE and the TNF-receptor;
  - b) genetically altering the cells with the polynucleotides to be screened;
  - c) cloning the cells genetically altered in step b); and

d) identifying clones that enzymatically release the receptor at an altered rate.

25. A method for screening substances for an ability to affect TRRE activity, comprising the steps of:

- a) incubating cells expressing TNF receptor with a polypeptide according to claim 9 in the presence of the substance;
- b) incubating cells expressing TNF receptor with a polypeptide according to claim 9 in the absence of the substance;
- c) measuring any TNF receptor released from the cells in steps a) and b); and
- d) correlating an increase or decrease of the receptor released in step a) relative to that in step b) with an ability of the substance to enhance or diminish TRRE activity.

26. Use of a polypeptide according to any of claims 7-8 or 11-12, in the preparation of a medicament for treatment of the human or animal body by surgery or therapy.

27. Use of a polynucleotide according to any of claims 1-3, 6, or 15 in the preparation of a medicament for treatment of the human or animal body by surgery or therapy.

28. Use of an antibody according to claim 16, in the preparation of a medicament for treatment of the human or animal body by surgery or therapy.

29. Use of a polypeptide according to any of claims 7-8 and 11-12, a polynucleotide according to any of claims 1-3 and 15 or an antibody according to claim 16, in the preparation of a medicament for treatment of a

disease selected from the group consisting of heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, and sepsis.

30. A method of treating cancer in a subject, comprising increasing signal transduction from TNF into cells at the site of the cancer in the subject according to claim 22 or 23.

31. A method of treating a disease selected from the group consisting of heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, and sepsis, comprising decreasing signal transduction from TNF into cells at the site of the disease in the subject according to claim 21 or 23.

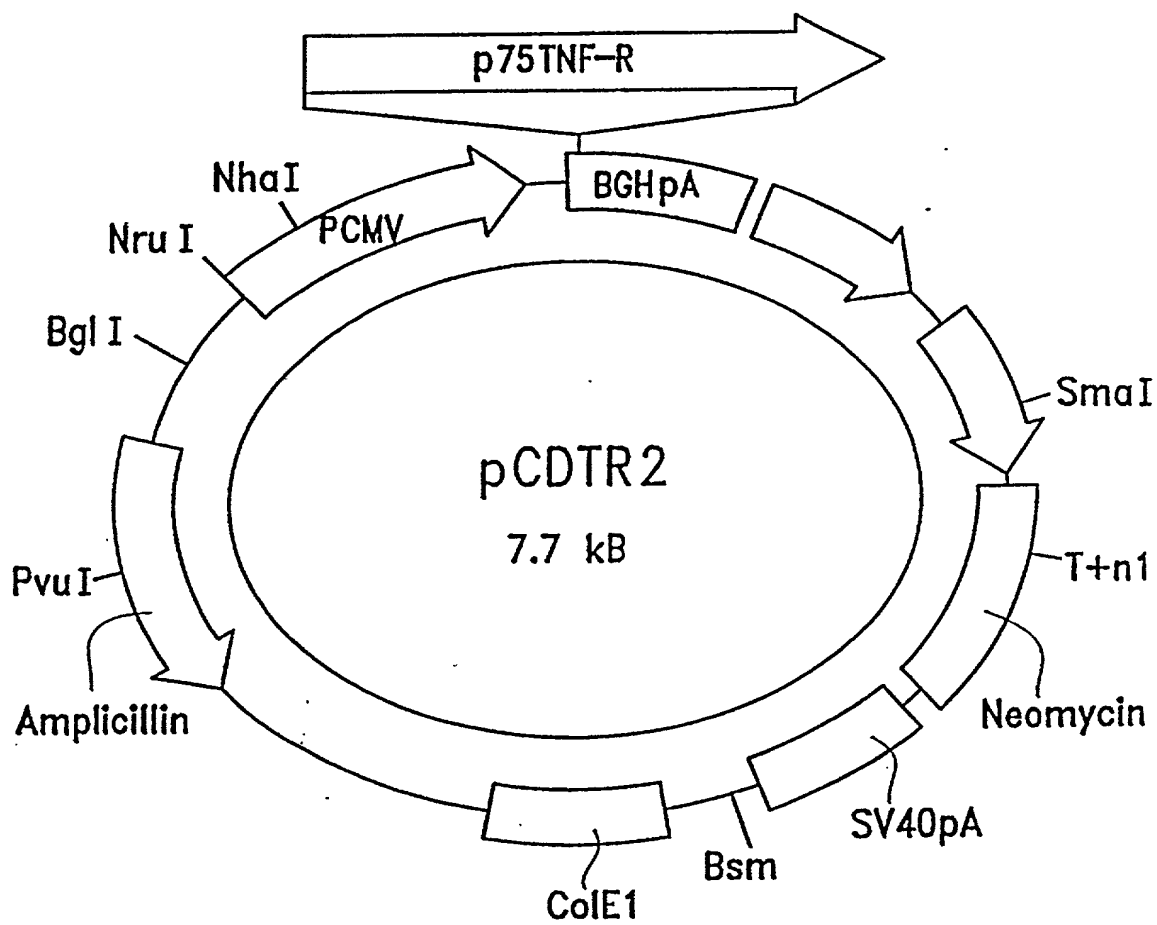
32. The method of claim 31, comprising administering to the subject an effective amount of the polypeptide of any of claims 7-8 or 11-12.

## ABSTRACT OF THE DISCLOSURE

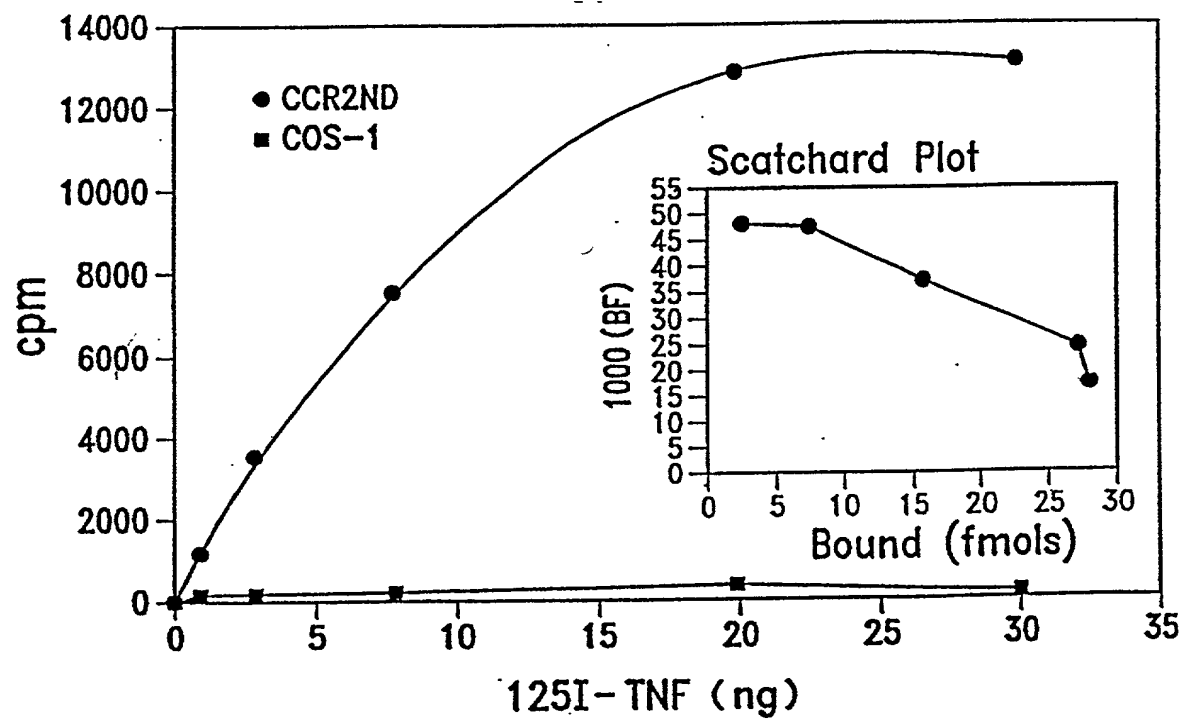
The biological effects of the cytokine TNF are mediated by binding to receptors on the surface of cells. This disclosure describes new proteins and polynucleotides that promote enzymatic cleavage and release of TNF receptors. Also provided are methods for identifying additional compounds that influence TNF receptor shedding. As the active ingredient in a pharmaceutical composition, the products of this invention increase or decrease TNF signal transduction, thereby alleviating the pathology of disease.

10

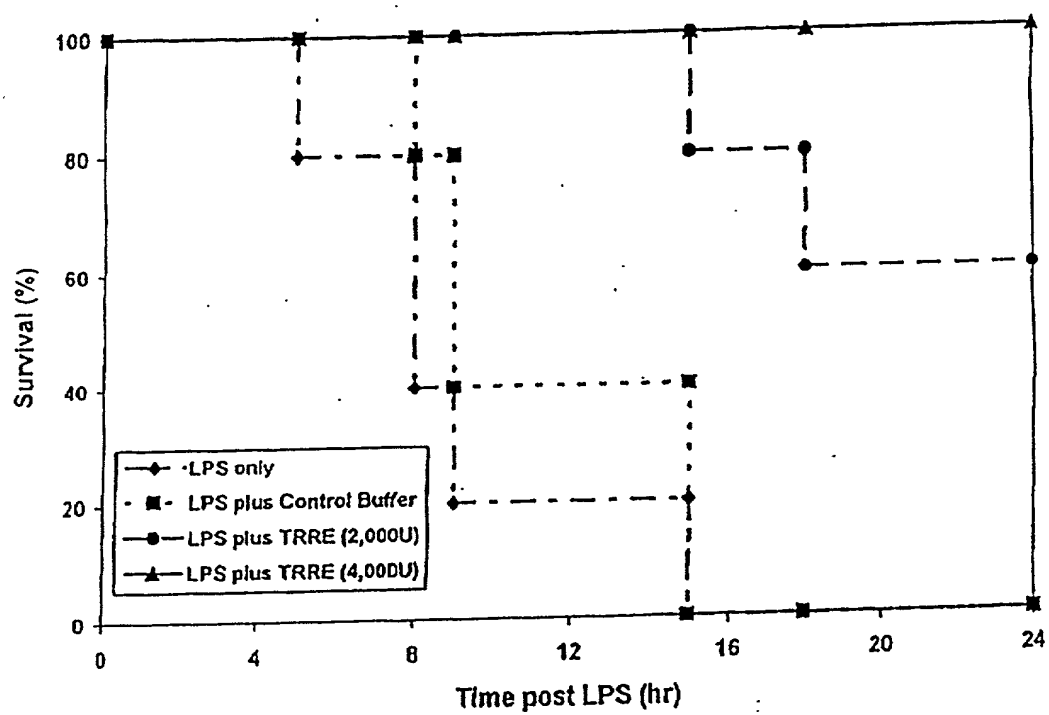
**Figure 1**



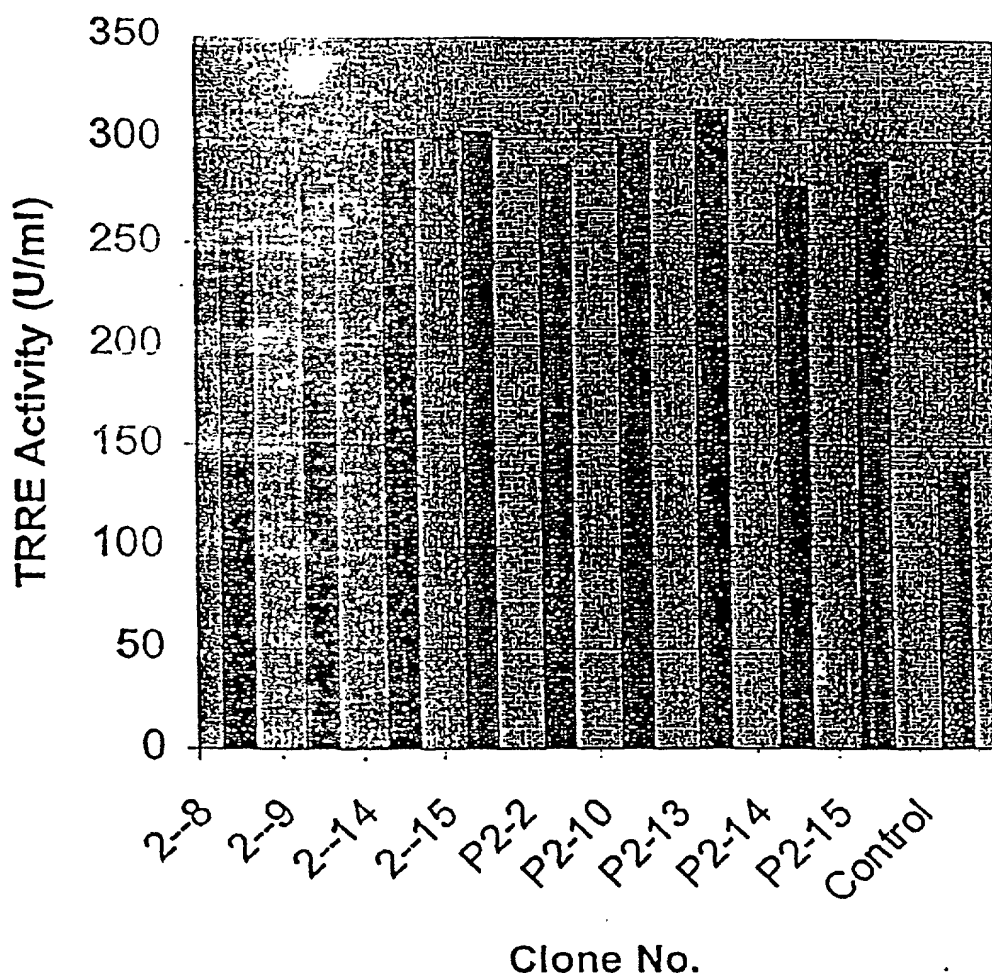
**Figure 2**



**Figure 3**



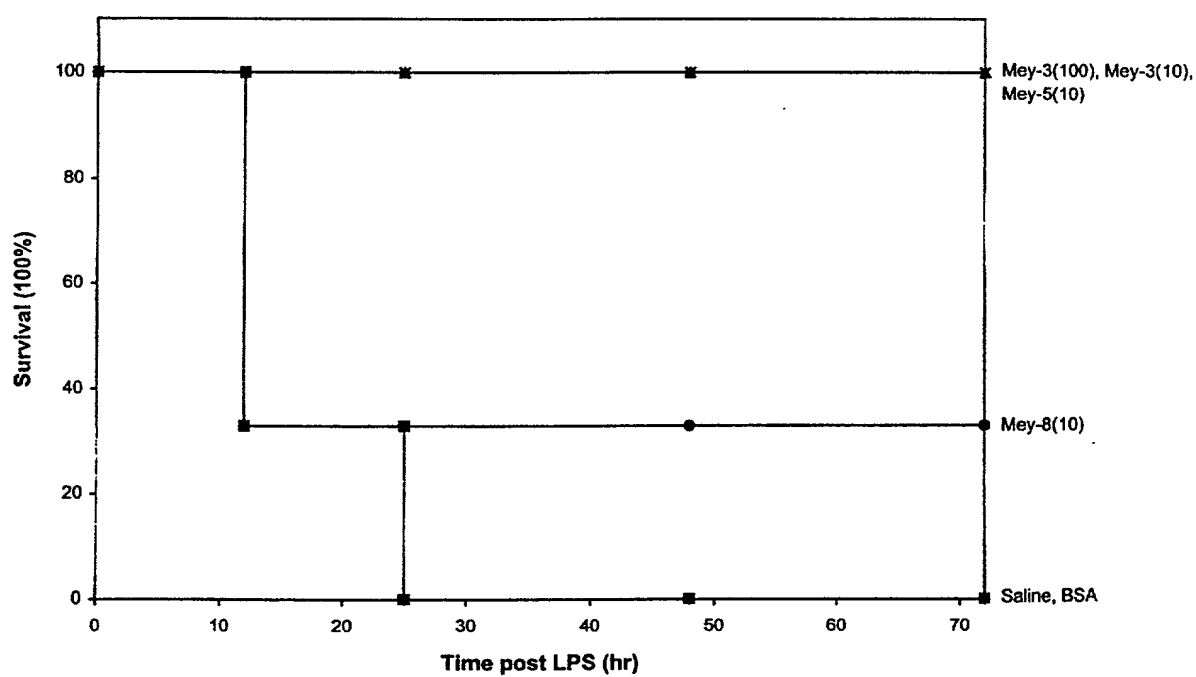
**Figure 4**





**Figure 5**

**Treatment of Mey proteins to prevent from mortality induced by LPS plus  
D-Galactosamine**



**DECLARATION FOR UTILITY OR  
DESIGN PATENT APPLICATION  
(37 CFR 1.63)**

☒ Declaration Submitted with Initial Filing      OR      ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e)) required)

<b>Attorney Docket Number</b>	IRVN-007CON
<b>First Named Inventor</b>	Gatanaga, et al.
<b>COMPLETE IF KNOWN</b>	
<b>Application Number</b>	N/A
<b>Filing Date</b>	Herewith
<b>Group Art Unit</b>	N/A
<b>Examiner Name</b>	N/A

**As a below named inventor, I hereby declare that:**

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Factors Affecting Tumor Necrosis Factor Receptor Releasing Enzyme Activity

the specification of which:

☒ is attached hereto

OR

☐ was filed on \_\_\_\_\_ as United States Application Number or PCT International Application Number \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined by 37 CFR 1.56.

Insofar as the subject matter of each of the claims of this application are not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designating at least one country other than the United States of America, listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) having a filing date before that of the application(s) of which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
	PCT		<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application(s) designating the United States of America, listed below.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
09/081,385	05/14/1998	
PCT/US99/10793	05/14/1999	

NOV-13-00 MON 09:17 AM BOZICEVIC FIELD&amp;FRANCIS , FAX NO. 650 327+3231

P. 04

Atty Dkt No. IRVN-007CON

DIRECT ALL CORRESPONDENCE TO:					
Name	Carol L. Francis				
Address	BOZICEVIC, FIELD & FRANCIS LLP				
Address	200 Middlefield Road, Suite 200				
City, State, Zip	Menlo Park, CA 94025				
Country	U.S.A.	Telephone	650-327-3400	Facsimile	650-327-3231

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

## Name of Sole or First Inventor:

Given Name (first and middle (if any))				Family Name or Surname			
Tetsuya				Galanaga			
Inventor's Signature						Date	
Residence: City	Irvine	State	CA	Country	USA	Citizenship	JAPAN
Post Office Address	77 Wellesley						
City	Irvine	State	CA	Zip	92612	Country	USA

## Name of Second Inventor:

Given Name (first and middle (if any))				Family Name or Surname			
Gale A.				Granger			
Inventor's Signature	<i>Gale A. Granger</i>					Date	13 NOV 00
Residence: City	Laguna Beach	State	CA	Country	USA	Citizenship	USA
Post Office Address	31562 Santa Rosa						
City	Laguna Beach	State	CA	Zip	92651	Country	USA

**DECLARATION FOR UTILITY OR  
DESIGN PATENT APPLICATION  
(37 CFR 1.63)**

☒ Declaration Submitted with Initial Filing      OR      ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e)) required)

**Attorney Docket Number**      IRVN-007CON

**First Named Inventor**      Gatanaga, et al.

**COMPLETE IF KNOWN**

**Application Number**      N/A

**Filing Date**      Herewith

**Group Art Unit**      N/A

**Examiner Name**      N/A

**As a below named inventor, I hereby declare that:**

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Factors Affecting Tumor Necrosis Factor Receptor Releasing Enzyme Activity

the specification of which:

☒ is attached hereto

**OR**

☐ was filed on \_\_\_\_\_ as United States Application Number or PCT International Application Number \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined by 37 CFR 1.56.

Insofar as the subject matter of each of the claims of this application are not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designating at least one country other than the United States of America, listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) having a filing date before that of the application(s) of which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
	PCT		___ ___	___ ___	___ <u>X</u>

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application(s) designating the United States of America, listed below.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
09/081,385	05/14/1998	
PCT/US99/10793	05/14/1999	

NOV-10-00 FRI 04:37 PM BOZICEVIC FIELD&amp;FRANCIS FAX NO. 650 327+3231

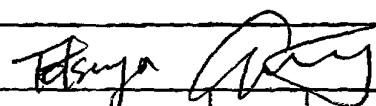
P. 06

Atty Dkt No. IRVN-007CON

DIRECT ALL CORRESPONDENCE TO:					
Name	Carol L. Francis				
Address	BOZICEVIC, FIELD & FRANCIS LLP				
Address	200 Middlefield Road, Suite 200				
City, State, Zip	Menlo Park, CA 94025				
Country	U.S.A.	Telephone	650-327-3400	Facsimile	650-327-3231

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**Name of Sole or First Inventor:**

Given Name (first and middle [if any])				Family Name or Surname			
Tetsuya				Gatanaga			
Inventor's Signature						Date	11/13/00
Residence: City	Irvine	State	CA	Country	USA	Citizenship	JAPAN
Post Office Address	77 Wellesley						
City	Irvine	State	CA	Zip	92612	Country	USA

**Name of Second Inventor:**

Given Name (first and middle [if any])				Family Name or Surname			
Gale A.				Granger			
Inventor's Signature						Date	
Residence: City	Laguna Beach	State	CA	Country	USA	Citizenship	USA
Post Office Address	31562 Santa Rosa						
City	Laguna Beach	State	CA	Zip	92651	Country	USA